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Proceedings of the Scientific Review Meeting on the U.S. Environmental Protection Agency Diesel Emission Health Effects Research Program Held at the Pentagon City Quality Inn, Arlington, Virginia on December 12-13, 1978

Geomet, Inc., Gaithersburg, MD

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This report is the proceedings of a scientific review meeting of the ongoing diesel emission health effects research program. The proceedings include formal presentations by Agency investigators and discussions of these presentations by those in attendance. Written comments on the program received after the meeting are also included. Expressions of a select scientific review panel are presented concerning the utility of the current research program to contribute significantly to the information base needed in making an adequate assessment of risk to the public health from an increased number of diesel-powered automobiles.

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PROCEEDINGS OF THE SCIENTIFIC REVIEW MEETING ON THE U.S. ENVIRONMENTAL PROTECTION AGENCY DIESEL EMISSION HEALTH EFFECTS RESEARCH PROGRAM

DECEMBER 12–13, 1978

Held at the
PENTAGON CITY QUALITY INN
ARLINGTON, VIRGINIA 22202

Health Effects Division
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C. 20460

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FOREWORD

Much of the current transportation effects research of the Environmental Protection Agency now conducted by the Office of Research and Development is targeted toward providing, by the fall of 1979, an assessment of the cancer risk associated with an anticipated increased use of diesel engines in light-duty vehicles. The importance of this assessment is emphasized by the fact that automobile manufacturers will soon be making major investment decisions as to whether diesel engines will power a significant portion of new automobiles in the 1980's. The Diesel Emission Health Effects Research Program Meeting was held against this background.

The purpose of this meeting was twofold: first, it was to present the ongoing and planned EPA Diesel Emission Health Effects Research Program to the scientific, industrial and other interested communities for their information, and allow EPA in return to receive their comments and concerns on the program. Second, the meeting was to provide a mechanism for an evaluation by a select scientific review panel of the program utility for contributing to the information base needed to provide an adequate assessment of risk to the public health from an increasing use of diesel-powered passenger cars.

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PROGRAM

Tuesday, December 12, 1978

Introductory Remarks
Jack Keeve, EPA/Health Effects Division

Welcome & Research Plan Overview
Roger Cortesi, EPA/Chairman, Mobile Source Resources Committee

Program Office Requirements
Stan Blacker, EPA/Office of Air, Noise & Radiation

Risk Assessment
Roy Albert, EPA/Chairman, Cancer Assessment Group

In Vitro Studies
Joellen Huisingh, EPA/Health Effects Research Laboratory/
Research Triangle Park, NC

Skin Painting Studies
Stephen Nesnow, EPA/Health Effects Research Laboratory/
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Intraperitoneal Injection Studies
William Pepelko, EPA/Health Effects Research Laboratory/
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Inhalation Studies
Kirby Campbell, EPA/Health Effects Research Laboratory/
Cincinnati, OH

Intratracheal Instillation and Injection Studies
Alan Shefner, IIT Research Institute
Clinton Grubbs, IIT Research Institute

Epidemiologic Studies
Robert Miday, EPA/Health Effects Research Laboratory/
Cincinnati, OH

Wednesday, December 13, 1978

Open Comment & Question Session

SUMMARY OF THE
SCIENTIFIC REVIEW PANEL

The Scientific Review Panel convened on December 13, 1978, to make its initial assessment of the current EPA Diesel Emission Health Effects Research Program following one and one-half days of presentations and discussions. A stenographic record was made of the panel's discussion. This summary, incorporating changes and recommendations provided by the panel members, has been developed by GEOMET, Incorporated from the original record.

The role of the panel was to identify and discuss the key issues raised during the meeting in relation to the following charge:

> Does the EPA Diesel Emission Health Effects Research Program, as described in this meeting and in the protocols given to the panel, provide an adequate basis for assessing the risk to public health from diesel emissions? If not, what data can be expected by Fiscal Year 1980 and how can the current program be improved?

Panel members agreed that a formal "peer review" of the EPA program could not be made on the basis of the material presented, and that they neither endorsed nor faulted the current EPA diesel health effects research program.

The panel recognized that EPA requires, for policy determinations, some basis for risk assessment prior to the end of 1979. The major concerns of the panel may be grouped in these principal areas:

- Exposure/Dose Relationships
- Characteristics of Exposure Materials
- Epidemiology, Dose-Response, and Other Related Questions
- Evaluation of Results from a Multiplicity of Experiments
- Other Problem Areas.

Exposure/Dose Relationships

Essentially all of the current EPA diesel emission health effects research programs consist of in vitro and in vivo experiments and model systems. The panel, recognizing the importance of relating exposures to doses in test systems and in humans, test system doses to human doses, and human doses to risk assessment, agreed that there is a need for better understanding of exposure/dose-response relationships.

Inadequacies in the current research include:

- Information on the elution, under physiologic conditions, of hydrocarbons from the surfaces of diesel carbonaceous particles

- Information on the regional deposition of inhaled submicron diesel emission or similar particles (surrogate aerosols) in man and laboratory animals
- Relationships of dose from skin painting and injection studies to dose under inhalation conditions
- Use of "marker compounds" known to have high biological activity as dosimeters in animal studies to estimate dose to critical tissues.

Characterization of Exposure Materials

EPA laboratory studies of the health effects of diesel emissions use specially prepared exposure materials intended to reproduce significant aspects of diesel emissions as they would impact upon human populations exposed to exhausts from diesel engines. The panel was concerned with the adequacy of present knowledge and use of diesel emission characteristics in health effects studies. Identified concerns of the panel were:

- The temporary cessation of EPA studies on the chemical and physical characterizations of diesel emissions.
- Significant health effects of diesel emissions may be overlooked because synergistic effects resulting from combined effects of atmospheric toxicants or personal habits (e.g., smoking) are not presently being considered.
- Solvent extracts from diesel particles may not be representative of hydrocarbon species found on the surfaces of particles inhaled by man or laboratory animals.
- Mutagenicity or oncogenicity results from studies using solvent extracts may have questionable relevance to human health risk associated with airborne diesel emissions.

Areas identified for further research were:

- Use of simulated serum suspension of particles for testing
- Development of an understanding of diesel emission chemistry, i.e., local interactions and those which occur during atmospheric transport
- Study of the behavior of diesel particles in animal models using "surrogate"-labeled aerosols with specified characteristics.

Epidemiology, Dose-Response and Related Questions

The panel expressed its opinion that a significant determinant of risk assessment of the health hazard from diesel emissions would have to be based on epidemiologic studies and human population data, but recognized that time constraints make it impossible to rely upon epidemiology for results in 1979. Concerns of panel members were:

- The only available community study of diesel emission health effects, conducted in London, can probably not be mined further for information which would prove useful to EPA policy-setting.
- Recognizing resource constraints, it is logical that major program emphasis has been placed on carcinogenicity in the current EPA program. In planning for future research, non-neoplastic health effects should also receive consideration.
- The relative risk of diesel emissions versus gasoline emissions should be considered through comparative studies (laboratory or population-based) using both classes of exposure and the same test systems.
- More attention needs to be paid to human exposure environments, perhaps through ambient sampling in high (e.g., bus terminals) and low exposure areas in the real world.
- Evaluations of risk may be based on possibly inappropriate models for extrapolation and response (e.g., tumors from skin painting as indicative of effects from inhalation).
- Toxicity of diesel emission extract materials is known, but toxicity of diesel emission is not yet well understood.
- Since risk assessment is an iterative process, a final answer cannot be anticipated upon conclusion of current studies. Therefore, a long-term research program of laboratory and population studies is needed.

Evaluation of Results from a Multiplicity of Experiments

The EPA research program includes a multiplicity of experiments from which a single answer is desired: that is, to what extent are diesel emissions a hazard to public health? The panel agreed that a major question is EPA's handling of a diverse body of experimental information to support a single coherent set of conclusions. Concerns of panel members were:

- A positive result in a definitive study of one type may not be prima facie evidence of carcinogenicity if it is offset by a negative result in a definitive study of another type.

- A hedge is needed to relate results of one type of study to those of another type of study, so that evaluation of risk can be made from mixed results of positive and negative studies.

- The data from the single set of exposure conditions being employed in hamster and cat studies may be inadequate to determine the characteristics of dose-response relationships.

- Fundamental information on the behavior of inhaled diesel emissions could be extremely valuable in the reconciliation of conflicting experimental results.

- Problems inherent in making risk assessments on the assumption of a linear dose-response curve through the point of origin must be recognized in evaluating a multiplicity of test results.

- Simplifying assumptions (such as an inviolate stoichiometry between exposure and dose from system to system) may be necessary to make decisions in 1979, but frameworks for making data more compatible at different levels of simplification are needed.

Other Problem Areas

The panel expressed concern about the quality control aspects of the studies currently underway, and suggested that consideration be given to developing methods by conducting blind analyses of laboratory specimens to avoid systematic bias and to ensure that the results of the studies have credibility in the scientific community. Formalized procedures for quality assurance should include means of ensuring consistency in chemical form, blind toxicologic assessment and, probably, a central point for the cataloging and distributing of all test materials.

The panel also expressed concern that, although the problem facing EPA is the relative risk to public health from diesel versus gasoline emissions, the question of risk from gasoline emissions is not being addressed in a comparable manner in the current EPA program. This could result in serious problems in establishing regulatory policy relative to diesel emissions.

Since it is evident that no new epidemiologic data will be forthcoming before the 1979 risk assessment must be made, it was suggested that a special panel of senior epidemiologists and statisticians be convened to evaluate presently available data and to make specific recommendations regarding long-term epidemiologic studies needed to provide information for an adequate risk assessment.

PROGRAM PRESENTATION SESSION
Tuesday, December 12, 1978

DR. KEEVE: Ladies and gentlemen, I think it's time to get started.

I'm Jack Keeve from the Health Effects Division in the Research and Development Office of EPA, and your Coordinator and one of the people responsible for organizing this meeting.

I want to make a few remarks having to do with procedural points and some of the format and details. I won't be giving the initial welcoming talk; Roger Cortesi, who is our general chairman, will be doing that. He will provide the formal welcome and technical background for the meeting. (Gives logistic details.)

We have a stenographic reporting service making a verbatim record of these proceedings. The stenographer can only report one speaker at a time so please, when you get to the question and answer period, step up the the microphone and identify yourself and make sure that your comments are heard by the reporter.

There are some handouts in the back of the room. If we run out, please put your name on the list we have provided to have them sent to you. Those of you that have come in and registered have already made out a card so that you will receive copies of the proceedings which we hope will be available by mid-January.

We have asked that your written comments or any additional papers, remarks or insertions that you wish to be made part of these proceedings reach our contractor, GEOMET, by the 27th of this month. We hope to have the final version available, cleared, and edited by the panel, myself and the editors by mid-January.

At this point I would like to introduce our general chairman, Dr. Roger Cortesi, who is the Chairman of the EPA Mobile Sources Research Committee, and he will then conduct the principal part of the meeting.

Dr. Gary Flamm from the Food and Drug Administration will be the general chairman, interlocutor, and leader of the panel itself.

Dr. Cortesi--

DR. CORTESI: Good morning. I'm glad that diesels got everybody started and here today on a bright and chilly morning.

I would like to go over briefly what we are trying to do at this meeting here today because we have a lot to do, and if we stick to the point we're more apt to get it done than if we don't.

The purpose of this meeting is to present in a public forum, so that everybody sees what is going on, what the EPA health research approach is to estimating what the risk that a fleet of light-duty diesel motor vehicles will present to public health. The program is designed for that purpose. It is not designed to open up new structures to understanding cancer or anything else. It is an applied health effects research program and should be considered in that light.

It is also not the purpose of this meeting to discuss what general EPA, or indeed United States Government, policy should be towards the diesel automobile, and that is outside of the jurisdiction of the scientific part of EPA. That is a program office function and we are nowhere near coping with that problem in EPA. The individual presentations, which you will be getting to try and fulfill this function, are by individual EPA researchers who will describe what they are doing, why, and, where appropriate, any results to date. The panel will then ask questions, discuss, and just generally see whether what we have proposed and are doing could be improved upon or whether it seems all right and move on, and from this moving through the research program, the final report that we will get will indeed condense the results of this meeting into any changes that might be both desirable and possible, and Dr. Flamm will be the ringleader for getting that done.

There are two speakers, as you can see, following me in this introductory phase. The first one will be Stan Blacker.

Stan Blacker, of the Office of Mobile Source Air Pollution Control, will give you an understanding of what the problem is that EPA is facing in this diesel question so that you will be able to put the scientific and the health effects research into the overall regulatory perspective.

After that Dr. Roy Albert, who is Chairman of the EPA Cancer Assessment Group, will describe to you the overall research strategy, the results of which will feed into his group for the producing of risk assessment numbers.

Stan--

MR. BLACKER: I am representing Mike Walsh who is the Deputy Assistant Administrator for Mobile Source Air Pollution Control, and I am his senior technical advisor.

Because of the auto industry's needs to meet stricter fuel economy standards and its desire to continue offering to the public a variety of auto body sizes, the industry is on the verge of dieselizing which should substantially increase the number of diesel powered automobiles on the American roads.

Small capital investments have been made by industry, but the major industry decisions, estimated to be a couple of billion dollars over the next couple of years, are scheduled to begin in early January 1980. Prior to this time, industry's interest in dieselizing can be reversed with little or no impact.

The Office of Mobile Source Air Pollution Control and the Administrator recognize that the pivotal issue on whether to permit the dieselization is the potential adverse health effects to the public that might result from the large-scale dieselization.

The health effects program that will be reviewed today will provide a portion of the health effects information needed by the Administrator as he makes his important, far reaching decisions on diesels, beginning as early as the summer of 1979. The remainder of the information will be provided by the manufacturers as they fulfill the requirements under 202(a)(4) of the new Clean Air Amendments.

There are a few other points I would like to make. The first point is that this panel and the research program in general should address two fundamental questions as I see it. The first one is to identify the important issues that must be addressed in this whole research program, and then to determine what research to perform to answer the questions and issues that are raised.

This panel must constantly be aware, I believe, that the health effects research program that will be undertaken is primarily structured around providing to the Cancer Assessment Group the critical information it needs to perform a human risk assessment. The experiments that are identified here are principally based on the Cancer Assessment Group's reliance on relative potency information as the backbone of its risk assessment. Assuming that there is a major acceptance of this approach, or at least an acceptance that there isn't another better approach available, the scientific panel should scrutinize the experiments that are presented here to ensure that the critical experiments being conducted will provide useful data needed to perform this risk assessment evaluation that the Cancer Assessment Group is planning.

As we see it, the principal objectives of the Health Effects Research Program is to provide hard data to the Program Office and to the Administrator for their evaluation of the diesel health effects problem. We are not principally interested in research for the sake of research. We are interested in obtaining hard data from that research which we can utilize in the risk assessment and in our evaluation beginning, as I said, as early as the summer of 1979; the ultimate question being whether the Program Office should recommend to the Administrator that certificates of conformity be issued under the various provisions of Title 2 of the Clean Air Act for diesel-powered vehicles.

That is all I'd like to say right now. At various times during the program, if the opportunities arise, I would be more than happy to discuss these and other points more fully and would be willing to speak to individuals, also.

Thank you.

DR. CORTESI: Thank you, Stan. Our next speaker is Dr. Roy Albert, NYU, who is Chairman of the EPA Cancer Assessment Group.

DR. ALBERT: Thank you, Roger. From the tone of what has been presented so far, it looks as if the CAG is going to take the blame for whatever comes out of this enterprise, but that has happened before.

I thought that I would spend a minute or two telling you about the CAG and how it came to be and what it is doing, and then spend a few minutes describing the perspective that we have of this problem and what might be expected from the kinds of data that are going to be produced.

The Carcinogen Assessment Group got started about 3 years ago during a period when the Agency was under considerable stress about its cancer policy—even more so than now. This was the era when the decisions about heptachlor and chloradane had just come out, and on a background of the decisions on DDT and aldrin and dieldrin, and there was a considerable amount of apprehension in the industrial and chemical and agricultural community about the EPA regulatory actions against carcinogens.

At that time EPA came out with something called "Cancer Principles" which seemed to reduce carcinogenesis down to a few declarative sentences, and this provided the basis for a considerable amount of criticism directed at the Agency in terms of its not knowing anything about carcinogenesis and having a half-baked approach to cancer.

At that time the decision was made to focus the Agency's policy on the risk assessment process both from a standpoint of the carcinogen risk assessment, and also to the socioeconomic impact assessment. As part of that enterprise we developed a guideline for the assessment of carcinogenic risk which took the position that the risk assessment aims at answering two questions.

The first--and I might say that this is a characteristic assessment of any risk--namely, the broader question, is how likely is the accident or occurrence to happen; and the second is if it does, what are the consequences? There are two sorts of separate issues involved here.

So that in terms of the carcinogenic risk assessment, there are two questions. One is how likely is the agent to be a human carcinogen, and the other, if it's assumed to be a human carcinogen, how much cancer is likely to be produced, given the exposure estimates with or without regulations?

Now the approach that has been taken in these guidelines is that they don't make a hard, black and white judgment about carcinogenicity. We look at the weight of evidence and make some judgment about the likelihood of the agent being a carcinogen, and obviously this--the strength of the evidence which is looked at as a warning signal that an agent could be a carcinogen--can vary from very strong where one has a solid body of epidemiologic evidence, that is multiple studies of human population, backed by animal data, and here the qualification is important. The multiplicity of the epidemiologic evidence is important because people are exposed to an awful lot of different things and it is when one has a similar pattern of response under different circumstances with a common denominator in the form of the agent in question that the more that one has, the stronger or the more force that the evidence has. The additional backup by animal data is also important in order to make really clear that the agent in question is the one that is of concern.

So on one extreme the warning signal comes through loud and clear. At the other extreme, and unfortunately the CAG seems to be involved--the bulk of the CAG's work is involved in this end of the spectrum; namely, that the signals come through pretty thin and murky. That is where one has a marginally positive response in one sex of one strain in a series of studies.

So in effect, from a quantitative standpoint, we make a judgment as to how likely the agent is to be a human carcinogen giving the approach on the weight of evidence.

The quantitative aspect, that is how much cancer is likely to be produced, is, I think, a somewhat controversial area because the uncertainties of quantitative extrapolations are probably even greater than those of qualitative extrapolations from animals to humans because we have two things to be concerned with. One is the degree to which one can extrapolate from animal responses to human responses, and the second is, and even more importantly, our ability to extrapolate from dose levels that produce appreciable responses in the usual population size that we deal with in terms of animals, or even in epidemiologic studies, down to levels of risk which are in the order of one in ten thousand, one in a hundred thousand, one in a million.

These are the kinds of risk levels that we are interested in controlling too, but it is obvious that there isn't the remotest chance of getting direct evidence at levels of risk that low.

So that we need some sort of extrapolation model to go from the higher levels of responses where we can actually make observations to the lower levels where we are concerned about the risk from a practical standpoint. Now there are as many models for extrapolation from high levels to low as one wants to have; everybody's got his own. They produce a range of estimates which can vary by as much as three orders of magnitude, not so much at the higher levels of risk, but when one gets down to very low levels.

None of these extrapolation models is really strongly based in theory, and I would emphasize that it could only be in theory largely that one can base these extrapolation models. But for practical purposes, the linear nonthreshold dose response extrapolation model is the one that I think has the most solid basis in scientific fact and evidence, and also has the advantage of providing an upper limit estimate as the conservative model of estimation.

The basis for the linear nonthreshold is really essentially that there is a very close correlation between carcinogenesis and mutagenesis. The linear nonthreshold response is pretty well established for mutagenic action, particularly in small organisms where lots of them can be studied.

There is a degree of consistency in a number of epidemiologic studies in terms of the dose response being compatible with a linear nonthreshold, and that's about the long and short of it.

So the quantitative aspect of the risk assessment involves our using animal data, epidemiologic data, or a combination of both, essentially with a linear nonthreshold dose response, and given the exposure estimates we come up with an assessment of the consequences of lifetime exposure or partial lifetime exposure in terms of excess cancer incidence.

Now in many cases we are forced to use animal data as the basis for the extrapolations to lower levels. In some cases, even with negative epidemiological data, we can factor that in. For example, if an agent shows evidence of carcinogenicity in animal studies--for example, ethylene dibromide produced an 80 percent or so incidence of carcinoma of the stomach by intragastric intubation, and it is mutagenic and so it is pretty clear that it is likely to be a carcinogen for people, yet the exposures in people that we were concerned with were by inhalation, not by consumption. At least one facet of the issue involved inhalation of this particular agent.

We did have an industrial study that was done on a limited group of individuals who were involved with exposure over a substantial period of time. The exposures were at relatively low levels and the characterization of the cancer pattern in that group was that there wasn't any convincing evidence of any excess cancer.

So in this particular case we used the epidemiologic evidence to provide an upper limit of the level of risk because we knew the magnitude of exposure; on the assumption that the agent was a carcinogen based on the animal evidence, the human data provided us a benchmark so that we could peg the response at an upper limit. It couldn't be any greater than such and such.

I might say that this in the future is going to be one of the most important uses for epidemiologic data, particularly occupational, because even with a relatively small size population, the observations come in very handily in terms of providing some sort of basis for intercomparisons of humans and animals.

Of course if we have epidemiologic data, that is peachy, and this doesn't happen very often. We have had it with vinyl chloride and a couple of other situations, but even with the human data it still involves the issue of the extrapolation.

So this in effect is what the CAG is about, and what it has been doing now for two and a half years. We have carried out risk assessment on the order of 40 to 50 agents which involved detailed evaluations of the literature, as well as the quantitative aspects of the assessments.

I might say that you may turn your nose up at the uncertainties of the biological side of the affair, but I would call your attention to the large uncertainties in the exposure estimates, and in our experience this has been one of the weakest areas of the whole process.

I should also emphasize that we have made no bones about the accuracy of the quantitative estimates. We have said over and over again that the results are a crude, ball park estimate of the magnitude of the risks which may be useful in giving a feel for the overall magnitude of the problem; whether or not the problem is the largest public health issue that looms on the horizon.

That is the sort of use that we would hope the quantitative assessments are put to.

Now in the case of the diesel exhaust, the first question--namely how likely is the agent to be a human carcinogen--is not an issue simply because whenever one burns organic material, whether it is the leaves in the backyard or anything else, one produces carcinogens.

So to our way of thinking, the issue of how likely diesel exhausts are to be carcinogenic is a nonproblem. There is no question that there is a carcinogenic potential to these exhausts.

The central issue then is how much, either in an absolute sense or in comparison with what we have now; namely, the gasoline engine.

So that our approach in terms of doing the risk assessment will be made--at least our thinking is at the present--by a series of approaches. I hasten to add that we don't need any data to do this, as I will point out, but it would be nice to have and we are looking forward to the high degree of productivity of this research program and the elegance of the data that it produces.

But in effect we are thinking in terms of three approaches to the risk assessment. The first involves an intercomparison of the potency of diesel exhaust with agents that in effect we have done risk assessments for, agents that are known to be associated with cancer, and these include--in humans--three instances.

One is the coke oven emissions. We have clearcut evidence that people exposed, particularly on the tops of the coking ovens, have a very large excess incidence of lung cancer, and we have already dealt with the issue of the impact of the drifting of these emissions out of the plant sites into the general countryside, and the cityside too.

So that an intercomparison of diesel exhaust with coke oven emissions would give us a handle in terms of making assessments for the consequences of diesel emissions on the basis of the experience with coking ovens, and the intercomparison with the population studies that have been done on roofers would provide another basis for evaluation of the impact of diesel exhaust. These individuals are exposed to substantial levels of tar, aerosols and vapors, and there is some pretty fair epidemiologic evidence and quantitative epidemiologic evidence, and this is, I think, a caveat that is an important thing to bring out. We are looking at epidemiologic evidence where we have quantitative estimates of exposure.

The third area which we haven't really used before but which would be appropriate to use is cigarette smoke in terms of making an intercomparison of diesel exhaust with cigarette smoke and making estimates of the magnitude of the hazards on the basis of such an intercomparison.

Now that is essentially the first approach, and when I say we don't need any data I am being somewhat facetious, but one, as an initial premise, could assume that they all have the same potency and go ahead and make a risk assessment on that basis.

The second area would be direct extrapolation from animals using the approach that we have in a variety of instances; given dose response data in animals, we then would assume a linear nonthreshold dose response and do the extrapolation on that basis.

I emphasize, of course, that this is only one-half of it. We need the exposure estimates that go with it.

The third approach would be simply a direct comparison of diesel and automobile exhausts for potency, and here one could simply say, we don't know what the absolute magnitude of the effect of either is, but we can talk about a relative effect. That is, for example, if diesels are 10 times more potent on whatever basis one wants to come up with, than given the magnitudes of exposure to both, we could say whatever the risk is from one, from gasoline, diesels will be X times more or less important.

Now this is essentially our thinking at the present. If anybody has got some other ideas, we would be delighted to hear them. We are not kidding ourselves about the goodness of this sort of risk assessment, but take the opposite point of view; namely, the alternative. If we didn't do anything of this sort, what have we got left other than the fact that exhausts are carcinogenic, and one really doesn't have any ideas at all about how important a public health problem the emissions might be. Consequently, crude as the approach might be, I think it will probably do more good than harm.

So what you will be hearing about is the kind of research program that has been generated by the laboratory people, and I must say that we have had a series of fruitful discussions where we have hashed these things through and looked at them, turned them inside out and shaken them out from the standpoint of the kinds of information they might produce, and what other kinds of studies might be done that would be more or less contributory.

So you will hear a series of areas which has been identified as possibly producing useful results, and as indicated in the program, this involves mutagenesis and an in vitro transformation study on these various materials for comparative assessment of carcinogenicity and mutagenicity.

The mouse skin model will be used to evaluate these agents as initiators, as promotors, and as complete carcinogens.

The intraperitoneal pulmonary adenoma system which was developed years ago by Shimkin will also be used as a tool for evaluating comparative potency. The intratracheal hamster model developed by Saffiotti will also be used, as well as a series of inhalation studies using the mouse adenoma system as well as the hamster.

This essentially is what I have to say at this stage of the proceedings.

DR. CORTESI: Thank you, Roy. I've got an announcement which I should have made before. If you look at your program on day 2, it says from 8:00 until 12:00 we will have open comments and question session. I don't know how effective that would be, but I think if possible, we would like to get started on the panel deliberation and on writing that report earlier than after lunch.

We certainly will not shut anybody off but if possible we would like to finish up the public comments by 10:00, and if anybody has any thing they wish to submit, a written version of it would be gladly appreciated with a summary given by the speaker.

And anybody who wants to be here and listen to the panel discussion should plan to be here at 10:00.

I now will basically turn this meeting over to Gary Flamm and the panel. Our first speaker today will be Joellen Huisingh of the EPA Health Effects Research Lab in Research Triangle Park, North Carolina.

Before we get started though, would each member of the panel stand up or raise his hand and say who they are, their field of expertise, and their institutional affiliation, please. Let's start on my left.

DR. SELTSER: I am Ray Seltser. I am Professor of Epidemiology and Deputy Director of the Johns Hopkins Comprehensive Cancer Center.

DR. WILEY: I am W. R. Wiley, Manager of the Biology Department at Battelle-Northwest.

DR. LEWIS: I am Trent Lewis. I am Chief of the Experimental Toxicology Branch in the National Institute for Occupational Safety and Health.

DR. EPLER: I am James Epler, Unit Leader in Comparative Mutagenesis, Mutagenesis and Teratogenesis Section of the Biology Division, Oak Ridge National Laboratories.

DR. FLAMM: I am Gary Flamm. I am Associate Director for Regulatory Evaluation in the Bureau of Foods at the Food and Drug Administration.

DR. ZEIGER: I am Errol Zeiger. I am Acting Head of the Environmental Mutagenesis Test Development Program at the National Institute of Environmental Health Sciences.

DR. JONES: I am Bob Jones, Associate Director of the Lovelace Inhalation Toxicology Research Institute in Albequerque, New Mexico.

DR. GOLDMAN: I am Marvin Goldman, Director of the Radiobiology Laboratory, University of California, Davis.

DR. CORTESI: Thank you, gentlemen.

Joellen, it's your turn.

DR. HUISINGH: I would first like to introduce Dr. Ron Bradow who is the Chief of the Mobile Source Emissions Characterization Branch. I would like him to make some initial comments to you about the chemical/physical nature of diesel particles. Dr. Bradow foresaw the magnitude of the problem of characterizing diesel emissions and started working with our group to combine the capabilities of in vitro testing with chemical characterization to better understand the nature of the organics associated with diesel particles.

It has been very helpful to us as biologists to design the research program that we are going to present today, and really to understand the problem, to have some appreciation for the nature of diesel emissions themselves. Presuming that this may help the panel members also, I would like Dr. Bradow to tell you about diesel particles.

DR. BRADOW: Diesel engines make soot, quite a lot of it relative to what has been characteristic of gasoline engines.

When the exhaust of a passenger car comes out in the air, it is mixed intimately and quickly with the ambient air, cools, and all the gaseous and particle components come to ambient temperature fairly quickly.

In the case of the gasoline engine, especially a modern gasoline engine with an oxidation catalyst, there are very few particles. Those which do exist are mainly sulfuric acid mist.

The amount of higher molecular organic compounds present in a gasoline engine passenger car exhaust is extremely low.

With a diesel passenger car there is a substantial amount of carbon and higher molecular weight fuel components in addition to the gaseous material which goes out into the exhaust into the ambient air.

As the exhaust of either a truck or a diesel passenger car goes out in the ambient air, the organic material, the heavy ends, the higher molecular weight components of the fuel and oxidized fuel components tend to become adsorbed on the surface of the soot particles.

This process makes it, in a way, kind of easier to look at a diesel engine than a gasoline engine. In history we have had gasoline engines running on fuels which contain tetraethyl lead. Those engines produce a substantial amount of base particles, lead chlorobromide, primarily, and in those cases we can obtain or separate the gaseous components from the condensed phase component rather readily; obtain extracts, for example, of the higher molecular weight components in gasoline engine exhaust, just as we can diesel.

But in a modern catalyst car, this is really nearly impossible. There are very small amounts of material to deal with.

In terms of handling the gases from either diesel or gasoline engines, there are some serious technical problems involved with concentrating trace components of gaseous products. These occur because there are, in addition to the organic components present in the exhaust, substantial amounts of NO, NO_2, and sulfur oxides.

As a result, if we attempt, for example, to take a liquid or a coldtrap condensate of either a gasoline engine or a diesel engine exhaust, we find a substantial amount of organic compounds which were not present in the gas phase which occur because in a coldtrap, NO and NO_2 are concentrated. There is some nitric acid/nitronium formation, and as a result any olefinic components which might have entered the coldtrap are converted to compounds such as nitroolefin, nitroalcohols, and nitronitrate esters.

What I'm kind of driving at is that one reason why we get to the particular position we are right now in diesel engine research is that the carbons associated with the exhaust are a very convenient trapping medium. We can separate just by simple filtration the higher molecular weight organic components because they are sorbed on the carbon.

This is not possible with the modern gasoline engines generally. We can screw up a gasoline engine to do this, but we have been working to this point exclusively with the particle emissions from diesel engines.

One of the reasons why we think this is not such a bad thing to do is that there is some evidence, I believe, that particles can be trapped deeply into alveoli, and there is a very slow turnover time for these materials. Consequently, it may well be that particles present a greater risk than gases.

The point I'm trying to make here is that experimentally we have access now to diesel particles. Gasoline engine particles contain little, if any, organic matters.

May we have the slide, please? (Slide presentation.)*

To duplicate the process of how to reproduce in the laboratory the process of discharge of automobile exhaust, either gasoline or diesel, in the ambient air, we try to use an air dilution technique which maintains at a dilution ratio quite a bit higher than would be characteristic of discharge into the ambient air.

For example, a dilution tube procedure such as shown here, is very common in our work. We discharge the automobile exhaust into a mixing volume, an 18-inch diameter tube is very common, diluted about tenfold with clean, filter free, particle free, ambient air, and then we have access by filtration or by accumulated gas samples in variable volume reservoirs, plastic bags, to do a variety of chemical analyses on those gases.

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*Reproductions of the slides presented by Drs. Bradow and Huisingh can be found in "Application of Short-Term Bioassays to the Evaluation of Complex Mixtures." EPA 600/9-78-027, September 1978.

Can I have the next slide, please? (Slide presentation.)

In the case of diesel exhaust, about a year ago we ran through a rather simple-minded separation procedure as maybe a German natural products chemist in the early part of this century might have done to search for an active principal in the plant extract.

We simply separated the acid base neutral components and fractionated the neutral components into a few simple fractions such as a paraffinic hydrocarbon, aromatic hydrocarbon fraction, and then an oxygen-containing one.

In attempting to isolate the aromatic hydrocarbon fraction, we found that there were two observably different kinds of bands in silica gel chromotography, one which was fluorescent and one which was not.

The fluorescent band, we labeled TRN, transistion fraction, since we didn't know what was in it. Subsequently, we found by Ames-testing all the fractions, that the activity lay in only the most--the least polar of the oxygen-containing compounds, and in the TRN, or the transition fraction.

May we have the next slide, please? (Slide presentation.)

The kinds of compounds we find in both the OXY II, that is the least polar of the oxygen-containing compounds in the mixture, and in the TRN fraction, are generally shown in this board, except for one major component of the TRN fraction which I chose not to show on this particular slide.

Anthrone, benzanthrone, naphthols, dibenzofuron, and two amino fluorines have been found. All of these have alkyl derivatives.

The transition fraction also contains very substantial amounts of fluorinones, nine fluorinones. The majority of the compounds present in these relatively nonpoor oxygen containing fractions are ketones, and with some phenols, not very acidic ones.

Can I have the next slide, please? (Slide presentation.)

We have now gone far away from this separation scheme. We can get Ames active material in initial extracts, and by high resolution, high pressure, liquid chromotography, arrive at a single cut which contains all of the active material in diesel exhaust in a small band.

The dotted lines on these chromatograms--there are two back-to-back chromatograms, each about 40 minutes, which enclose within the dotted lines all of the active material that is present in diesel exhaust extracts.

This comprises only a few percent of the total particulates, perhaps as much as 10 percent of the extract.

What we are driving at here now is we can quickly get to fairly massive amounts of material from diesel exhausts to test. We cannot do this with gasoline engines. The absence of carbon in the exhaust of gasoline makes this sampling procedure totally useless.

In a few special cases we can produce soot in gasoline engines by running them abnormally; that is, for example, in a three-way car we can disconnect the oxygen sensor and cause the engine to run abnormally rich, produce some carbon and separate particles.

In the case of older cars burning leaded fuel, we can get some particles and some extract in those cases. But in the general case of a normally operating gasoline engine now, the organic yields are very low, so low as to be nearly unmeasurable.

DR. HUISINGH: I am sure that Ron will be willing to take questions and participate in a discussion when I finish the presentation of our program.

If I could have the first slide, please. (Slide presentation.)

Initially we began working with Dr. Bradow's group by applying short-term bioassays to the characterization of the organics from diesel particles. The purpose of this research was to use bioassays to direct the fractionation, and ultimately to identify any biologically active components in diesel exhaust.

We have made significant progress and I think it would be helpful in reviewing future plans to have a capsulation of the progress to date. The data I will discuss is available as proceedings of a symposium we conducted in Williamsburg on the "Application of Short-Term Bioassays to the Evaluation of Complex Mixtures."* That has all of the data that I will just very briefly summarize.

Our branch in the Health Effects Research Laboratory has been applying a phased approach to environmental screening of health effects shown on this slide.

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*EPA 600/9-78-027, September 1978.

At the first level, which we refer to often as the detection level, we use microorganisms, and often we do use the Ames salmonella typhimurium histidine reversion assay to detect unknown--when we are testing known compounds or complex mixtures to detect materials that are potentially both mutagenic and carcinogenic.

We also, depending on the nature of the material we are looking at, often use mammalian cells to look at the cellular toxicity of a material.

So the first approach we took, a little over a year ago now, was to apply basically these two test systems to the fractions that Ron showed you. I won't say a lot about toxicity because our initial findings--actually these were studies by Meltz at Southwest Research Foundation--that really none of these fractions were very cytotoxic. These are the fractions from the organic materials, the acids, bases, neutrals.

The acids I believe were a little more toxic than the others.

All of these fractions as Ron described were tested in all five tester strains where there was enough material in the salmonella assay, and I'm going to just very briefly tell you about that data, but it is all in here so if you want to know more about it, you can look in here, or if you need additional copies, you can ask me for them.

While I am on this slide, I would like to go on and before I show you any of the salmonella data, tell you that as Ron said we did find that most of the fractions did have some mutagenic activity, and most of that activity was direct acted and responded with the frameshift tester strain, which I will show you.

The mutagenic activity, even though it was concentrated in oxygenated neutral fractions, was also detectable in the total extracts.

So we began to take the material that was available to us at that time, which was primarily these most active fractions and the neutral fractions, and apply a battery of mammalian cell tests to see if we could detect point mutations in mammalian cells, primary DNA damage in mammalian cells, and oncogenic transformation.

These tests and microorganisms were with the total extract as well as the fractions which were identified were positive. The assays, the primary DNA damage assays--I want to give you a little feeling for this because some of the assays we have proposed to use in the future, and you may have questions, the panel may, why we have selected some assays and not others, I would like to tell you just a little bit about our experience to date with these materials.

Now there are, as you can understand, complex mixtures, and they have some difficulties in testing as compared to testing pure compounds.

The primary DNA damage assays in bacteria generally require diffusion away from a filter paper disk, and we found that the diesel organics did not diffuse properly, so therefore we felt that these were not appropriate tests to pursue.

We have not done to date very much with chromosomal effects. We have that in our plans for the future.

We have done a series of assays with mammalian cells for point mutations and for primary DNA damage. We have point mutation assays both in mouse lymphoma cells and in BALB cells and the preliminary results we have gotten back have all been positive.

We haven't actually summarized and reported out all of that data yet because some of the tests are being repeated.

The primary DNA damage assay, one assay that was conducted was unscheduled DNA repair in WI38 cells, and that was statistically negative and it appeared that it could be marginally positive if tested more thoroughly.

The mammalian cell tests that have been conducted on oncogenic transformation, which is an assay that we consider to relate more to carcinogenesis than--the mammalian cells treated with the chemical actually are transformed to cells that are considered to be morphologically transformed.

These tests, the preliminary results we have gotten back on the total extract as well as the most active fractions, have been positive, although there are some difficulties in running these assays with the complex organics from diesel. It has not always been possible to obtain dose response in this assay.

Based on the preliminary positive results, Dr. Bradow provided us with the remaining neutral fraction to initiate a skin carcinogenesis study in the Sencar mouse. Dr. Stephen Nesnow will describe this program and planned research.

Why don't you give me the next slide? (Slide presentation.)

Now I would like to tell you a little bit more in detail about the salmonella histidine reversion assay results that we have gotten with the diesel exhaust. Most of you are familiar with the salmonella assay. It involves bacteria which are in fact mutated, as the paper has reported, I think.

A combination of these bacteria with an activation system, and these assays have all been run with and without activation, the chemical is added, and the bacteria and the mixture is plated and incubated.

The studies are done in such a manner that we do get dose response data. We measure the spontaneous mutation rates and apply increasing doses of chemical to the plates so that we can quantitate the revertants.

Using this assay though, it is not possible to quantitate the survivors. We can just look at the plates and determine when we have toxicity, or look at the general dose response and tell when we have toxicity.

We have only just begun doing some work with the suspension assay in which we can quantitate survival, and therefore actually get mutation frequencies.

Next slide... (Slide presentation.)

I realize that this is a lot of lines, and all of this data is in that paper, but I just want to give you a quick feeling for the nature of the mutagenic activity.

These are plots of average numbers of revertants per plate verus concentration of fractions. This is the data from just two fractions, the transitional fraction that Ron mentioned, and the OXY fraction.

They are both subcuts of the neutral organics. These are the different tester strains that are employed, TA1538, TA1537, TA1535, TA100, and TA98 across the top.

The solid lines are with metabolic activation, and the dotted lines are without metabolic activation.

You can see in TA1535 that the activity is negative, or very marginally positive. It was positive here with one sample, and that would indicate, since we do see positive results in the frameshift tester strains, that the material we are dealing with probably is a large molecule like those that Ron showed that would be causing some type of a frameshift mutation and not a small alkylating region.

You can see especially in the tester strains that have been made more sensitive with plasma, TA100 and 98, that most of the activity is direct acting. In fact, these samples are all from heavy-duty diesel truck and bus engines.

But the recent samples we have been looking at from light-duty diesel vehicles show very similar results, and in TA100 we even see more activity with activation.

In 1538 we see more of the effect of activation than we do in any of the other tester strains, but if you look closely--it may be a little hard to see here--the initial slope of this line is not very much affected by the activation and it is really only out at the higher doses that the activation shows a significant effect. It may be that the activation is really detoxifying some components in there rather than activating the components.

The next slide, please. (Slide presentation.)

The current research involving short-term, in vitro bioassays has two major components.

The first component is the application of the microbial mutation assay to the evaluation of mobile source emissions. The second component involves the employment of a battery of mammalian cell mutation oncogenic transformation bioassays.

The first part of this program is employing a microbial mutation test and involves these six components, and I will give you a little more detail on each one of these. I think that the panel members have a document that listed these.

The first part is the development and application of techniques which is the main part of our in-house program as it applies to the complex mixtures, and specifically the diesel problem; in vivo exposure samples, fractionation characterization studies, which is really how we got into this with Ron Bradow's group, comparative matrix studies, mobile source evaluation studies, and ambient exposure studies.

The next slide? (Slide presentation.)

This tells you more details about that first part of the program.

We would like, if possible, to do some developmental work on sampling on the gas phase. I think Ron made a strong point about the problems we have with really looking at the biological activities of the diesel emissions that are not bound in particles, and that this really applies, of course, importantly to the gasoline situation also.

It is possible, and we have done some work in ambient air, to try and trap the gas-phase components and put them into bioassay, but at least to date I should indicate that we haven't been too successful in applying this to mobile source emissions.

There appears in many cases to be artifacts formed and we try to collect these materials.

We have done a number of studies jointly with Ron looking at different sampling methods with those particles. There has been a lot of controversy over whether the dilution ratio that has been employed is the appropriate dilution ratio; he has performed some studies that widely vary the dilution ratios. We conducted a bioassay on those samples where he has also looked at the organics.

We would like and are already embarking on some studies on extraction methods. A lot of the studies you will hear about today, and most of the work that we have planned with the mammalian cells and the rodent skin painting, will involve organic solvent extraction of the diesel particles.

I think that that is a very serious question, and I would like to come back to it at the end, as to whether those organics ever really get off the particles in man.

We have, with Ron's group's help, and Roy Zweidinger, who is here, been doing a solvent study looking at a wide range of organic solvents and their different capabilities to remove the mutagenic activity from particles.

We have done some work with looking at whether physiological fluids can remove this activity from the particles, and plan to do some cellular studies. We do a lot of work with the rabbit alveolar macrophage and have planned some studies combining engulfment of those cells in vitro and looking at release of mutagenic material.

We have done already a number of studies on storage and stability and this is important. I will show you a little bit of data from this.

We really have very little means except by some type of organic chemical screening method or the salmonella bioassay to do any quality control on the samples that will be used over quite an extensive period of time for skin painting and other whole animal studies, so right to this date we really don't have anything to rely on except this assay and on the organic analysis that can be done.

We hope to look at, and plan to look at a series of modifications of the salmonella assay. We do routinely do the suspension assay and hope to try maybe some direct exposure methods in the gas stream.

The data that will be coming out of this program with the salmonella assay is going to be very large. We have our own laboratory and several contract laboratories involved so we are working quite hard now in designing the quality control measures necessary for intralaboratory comparisons, comparisons of samples, quality assurance, and just the methodology for handling data and statistically comparing it.

The next slide, please. (Slide presentation.)

I just wanted to show you a few things that we have done to date related to this part of the program. This graph is also in that paper.

This is the effect of two different solvents on the mutagenic activity of diesel particles from a Volkswagen and a Mercedes. This is plotted average number of revertants per plate versus the milligrams of organic extracts.

Of course if you look out here where you begin to see toxicity, you do see some significant differences between the dichloromethane extracted material and the cyclohexane extracted material; however there is a smaller difference down here in the initial slope of the lines.

One important thing to keep in mind, and I will come back to it later, is the potential application of this assay to the comparison or evaluation of the mobile sources at this time. We feel it is pretty risky, and I will elaborate more on that later, but one risky point you can see right here is that depending upon the solvent you use to extract the particles, you preferentially get different amounts of mutagens, maybe different mutagens, we don't know how many are there, and depending on the solvent you select, the comparisons come out different.

Using the cyclohexane, we see a much bigger difference between the two vehicles than we do when we use the dichloromethane, and we don't know the significance of that, and it makes it pretty hard to draw conclusions when comparing vehicles or other engine parameters.

So I would like to leave the impression at this point that this is really pretty researchy and not really ready for routine applications.

The next slide? (Slide presentation.)

This shows you the storage study that was conducted. Ron's group produced diesel particles and then extracted them. We bioassayed them the very same day; they were stored either on the filter or stored as the extract and were bioassayed 8 weeks later.

This particular graph was plotted for a light-duty diesel vehicle, the Nissan. This was plotted as milligrams of particulate. Actually the particulate was not put on the plate, the organic extract was, but we calculated the milligrams of particulate.

This will give you a little feeling for where the mutagenic activity lies in terms of the milligrams of particulate and plotted versus the revertants for the average number of revertants per plate.

Again, if you look at the actual numbers in the paper, this initial slope of the dose response curve, we expect that toxicity is not playing as important a role. This initial slope is not greatly affected by storage.

However, you can see that these are the stored samples, these are the samples stored as the extract, and these are stored on the filters; that we do get an increase in toxicity and a resultant decrease in the slope of the line with storage.

What effects this has on carcinogenesis studies, we really don't know, but just based on this, we are trying to understand what we can to store the material on filters. This hasn't always been possible.

Next slide, please. (Slide presentation.)

The next part of this program is the application of in vitro methods, microbial mutagenesis, and also on this slide I have shown some other work that is not microbial mutagenesis, but really the in vitro support that we are hoping to provide to the HERL Cincinnati group. They will be here later to tell you about the whole animal inhalation studies.

We plan to look at mutagenic metabolytes in the urine and have performed chromosomal analysis on bone marrow and sister chromatidic change in vivo on lymphocytes.

We feel like this will begin to provide some information on the potential release of these mutagenic materials from particles in the in vivo exposure situation.

May I have the next slide? (Slide presentation.)

This is, of course, the part of the program that Ron described, the fractionation characterization studies being conducted by his group and our support to those studies. To date those studies have been primarily conducted with particles from heavy-duty bus and truck engines, and in the next year we hope to be able to provide support to them to push this area back further and hopefully be able to identify some potentially mutagenic compounds in light-duty diesel vehicles.

This involves conducting bioassays on major fractions, subfractions, and he begins to do GCMS and identify specific compounds, and his group obtains pure compounds or synthesizes them and we test the pure compounds, trying to get closer and closer to what the mutagens are.

We also have plans in the future to carry this to the actual ambient situation, and possibly ask the question, if we collect diesel particles in the ambient air, do we see the same materials?

The next slide. (Slide presentation.)

I'll say more about this later, and the comparison matrix study that Dr. Albert referred to, but we will, of course, first of all take these materials and do a complete salmonella typhimurium study with all the tester strains; the diesel emissions, coke oven emissions, tar, cigarette smoke condensate, and of course it has already been well characterized with benzo-a-pyrene.

The important thing that we will be able to do with the salmonella assay that may not be possible to do with any of the other assays that will be discussed today because of the problems that Ron described are comparisons to gasoline emissions. I should say here--well, let me go just a little bit further--home heating oil, as you know, uses number 2 diesel fuel oil, and we have plans underway to collect these emissions and have already conducted a number of ambient air studies and I will describe more of these later.

We have been able to bioassay some gasoline emissions; one from heavy-duty trucks, one from a catalyst vehicle operating as Ron described under malfunction, I guess, and in those cases we do see mutagenic activity and that mutagenic activity is in the same order of magnitude as we see with diesel emissions.

But it is a much harder problem to collect enough material to run these assays in the same dose response region, and it is for that reason that I think it is quite questionable whether we can do skin painting or mammalian cell assays with these materials.

We barely had enough to run one tester strain properly, and certainly not enough to even run all the tester strains.

Ron?

DR. BRADOW: Joellen, the experiments that we have done so far with one catalyst passenger car from which some material was derived, indicates about a fiftyfold lower emission rate of the organic fraction from the gasoline car compared to say the diesel passenger car. So it's a factor of nearly two orders of magnitude in the emission rate.

DR. HUISINGH: Okay. We do plan to conduct some mobile source comparison studies. We have been very interested in using short-term tests, not only to detect potentially carcinogenic and mutagenic material, but possibly to apply these bioassays to comparing process conditions in stationary sources to prioritized emissions for research purposes, and it is not at all clear at this time that these assays--certainly not now--and it is not clear that they could ever be used in the future for regulatory use to compare vehicles or control technology, fuels, or any of these other things listed. We feel it's worth taking a look at this and at least knowing what order of magnitude we're talking about.

We have done some work to date on comparing vehicles. We have done some work on comparing fuels that I can describe briefly. We are now currently looking at vehicles with and without catalysts, and we will be looking at some under different malfunction cycles.

The main problem I would like to emphasize here, in doing these studies, and especially in interpreting these studies, is we do not know what is the appropriate sample handling technique. We don't know which solvent we should really be using to extract these particles. We can define which solvent gets out the most mutagens, but whether that would be the most representative solvent to make comparisons is not clear.

I think the most serious question is interpretation of the data. If one vehicle puts out emissions that is more mutagenic in the Ames Test than another, does that really mean anything at all?

Ames has given recent presentations and published some studies that have been done comparing potency in the salmonella assay with potency in whole animal carcinogens. These studies have all been done with pure compounds, and have all been done primarily looking at order of magnitude differences.

This assay does detect six orders of magnitude differences in mutagenic potency, and it is possible to detect six orders of magnitude difference in carcinogenic potency. So far we see nothing like six orders of magnitude difference in any of these parameters.

If we are able to get a mutagenic response, and I will show you in the next slide the magnitude differences we see are much smaller than that, and what significance these small differences have are just not clear. (Slide presentation.)

This is a study from the emissions, particles from VW Rabbit and Mercedes using five different fuels. You can see the data is in revertants per plate per milligram of particulate and only the plus metabolic activation is shown here, and only one tester strain, which brings up another question.

The comparisons we see are different; with or without activation they are different in the different tester strains. We do see that the minimum quality fuel which is highest in aromatics and highest in nitrogen, does give the highest specific activity, but when you compare that with the jet A fuel or premium quality fuel, we see maybe four times more activity here and 61.4 and 153. We really don't see orders of magnitude difference. (Slide presentation.)

The next slide just shows again--we are finding though some things that we think are worth pursuing in the research mode, and that is trying to identify what are the materials in the fuel, what precursors in the fuel might give rise to mutagenic activity because potentially if you could eliminate those from the fuel, that would be a useful thing to know.

Here you see again the VW data, looking at the mutagenic activity with the different fuel types, and the minimum quality fuel shows the aromatic content, how much higher it is from the other fuel.

Also the benzo-a-pyrene analysis on the emissions shows that there is more BaP in the emission, although that BaP which is in anagrams per milligrams per particulate can in no way account for the mutagenic activity that we see.

The next slide, please. (Slide presentation.)

The last area of work in the microbial mutagenesis program is application of the bioassays to some ambient exposure situations where massive volume samplings will be taken in the field, and studies will be done to compare high diesel density areas with low diesel density areas, and the sites, at least primarily identified, have been in New York bus terminals, a high density area, and possibly some collaborative work on the Allegheny Tunnel, the low diesel density area. Of course there will be controls nearby for this site so there will be site control and also some work done in New York City's Central Park.

We are already doing this type of work where we take the massive volume samples to the field, we collect particles, extract the particles, and we can carry out mutagenesis testing on the total extract as well as also carry out fractionation characterization studies asking if the material that we identify in the diesel particle emissions, if the same fraction is mutagenic in the ambient air.

Of course, ultimately if you can identify what the mutagens were, and then can test them to know if they were carcinogens, you can then monitor chemically for those directly in the environment. But in lieu of that, we are proposing to collaborate with the environmental monitoring and support lab on these studies.

That is the conclusion of the microbial mutagenesis part of our program. (Slide presentation.)

The next slide shows the assays that will be employed in the confirmatory bioassay part of the program. These assays cover two main endpoints: mutagenesis and carcinogenesis.

The assays employed under mutagenesis are those detecting gene mutations. The ones we are employing are the mouse lymphoma assays, the BALB/C mouse embryo fiberblast assay, and the CHO assay.

We will be looking at chromosomal effects in the lymphocytes and bone marrow as I described, and possibly some in vitro chromosomal effects. DNA damage will be detected using unscheduled DNA synthesis and sister chromatid exchange, and the carcinogenesis in vitro studies will primarily be concentrating on the BALB/C mouse embryo fiberblast assay which we have used to date. The Syrian hamster embryo assay will probably also be employed.

The other assay that we have available in-house that Steve Nesnow conducted is the C3H10T and a half mouse embryo fiberblast assay.

The next slide, please. (Slide presentation.)

The next slide will show you the samples that we expect to look at with this confirmatory battery.

I'm going to show you two separate matrices, and Steve will also be referring to this and this has caused a little bit of confusion among us, those of us describing our programs. I hope this will make it clear.

I am going to show these matrices described as one, a diesel comparative matrix, and the other a source comparative matrix which has the coke oven in those samples.

This first matrix would just be diesel samples selected hopefully to represent the widest range of mutagenic activity that we can obtain in the Ames salmonella assay. It will include hopefully the emissions from Oldsmobile, Nissan, Turbo Rabbit, Caterpillar, which is a heavy-duty engine, and a Mercedes.

From work that Ron's group has already done, we do have preliminary data on several fuels, such that we think we can hopefully select samples and conditions such that we can get a range of activities in these samples.

We then plan to take these samples and carry out gene mutations in mammalian cells and oncogenic transformation in mammalian cells and skin carcinogenesis in mice. This would hopefully shed some light on what really is the range of activity we can see in the microbial mutation assays. Do we see that range of activity in mammalian cell tests in vitro, and do we see the same range in order of activity in rodents and any in vivo assay, and this in vivo assay Steve will describe in more detail, how those assays will be conducted.

The next slide, please. (Slide presentation.)

The next slide, which really includes more biological endpoints, will be the matrix that will compare the different sources, those sources which the Cancer Assessment Group has described as having human epidemiological data.

The diesel sample that we have the most of and will be employing is the Oldsmobile. The coke oven emission will be collected with a massive volume sample on top of a coke oven.

Roofing tar emissions will also be collected with a massive volume air sampler.

Cigarette smoke condensate is really not quite comparable. It is not an extract from a particle, but it is standard smoke condensate that has been used in other studies.

Benzo-a-pyrene will be used along with all the other appropriate positive controls, and these assays will all be conducted in a dose response fashion, and those particles for these assays and the exact number of doses are all described in a draft research plan, which I think all of you have.

The most important assays again will be those I described; the gene mutation assays in mammalian cells, chromosomal effects in mammalian cells, primary DNA damage in yeast and mammalian cells, and we do find this assay is positive with diesel extracts to date.

Carcinogenesis assays will be conducted in mammalian cells. Oncogenic transformation assays, and all of this part of the matrix, presuming that we get the samples within the next few months, which we expect, will be completed by the end of the summer.

The skin carcinogenesis assay that Steve will describe will be initiated and we will show you the schedule of when we expect to have data out of those.

Judy Graham and Don Gardner will tell you more about the intratrachael instillation studies, and forgive me for putting question marks here, but at the time I prepared this I didn't know for sure if we are going to be able to do all of these samples in the intratrachael instillation model.

Can you give me the next slide, please? (Slide presentation.)

I would like to summarize with this slide one of the questions that I think we are all wrestling with. I took the liberty of trying to look at the total program a minute and put some of the work we're doing in context with the total program and make up a little matrix on what kinds of samples we're going to be looking at, because I think that one of the things that I would certainly appreciate the panel members' comments on is what we can do in this problem area.

A lot of the work that we have planned will be done with organic extracts on animals, as will be described with skin painting in mammalian cells and in microorganisms.

Then the question comes up, do these organics--on which we have good evidence that they are mutagenic and carcinogenic at least in mammalian cells and microorganisms--ever leave the particles and get to the target cells when a whole animal is exposed by inhalation?

I will summarize for you here how we have planned to begin to address this question.

In the area of direct exposure, which I would presume is the most relevant exposure, you will hear later the inhalation studies from Cincinnati. We are working with them to do in vitro and other analyses on tissues and body fluids from exposed animals.

We have been able to expose the plant, Tradescantia, using the micronucleus test with whole diesel exhaust. Preliminary data show that as little as 10-minute exposure causes significant induction of micronuclei. We plan to continue these studies.

It is possible to directly expose mammalian cells and microorganisms to gaseous conditions. We would like to initiate work in this area since Dr. Bradow's mobile source emissions laboratory is in the same building in which we have cell culture and microbial laboratories. The collected particles themselves will be applied to animals, and you will hear about that in the intratracheal work. We plan limited studies with particles directly in mammalian cells.

To date, the collected particles themselves give negative results in the microbial assays, as you would expect, because the organics are tightly bound to the soot. I believe GM has reported some positive results under certain conditions.

Since the exposure time is very short, the chance of the organics eluting off the particles and reaching the DNA is not very likely. For this reason, we will continue to explore physiological and cellular extraction techniques. We consider the animal fluids and tissues from exposed animals to be more realistic physiological extractions.

Thank you.

DR. FLAMM: Let me begin by asking the panel members if any of them have any questions at this point.

DR. SELTSER: This addresses something which I didn't see in the material we got beforehand, and it really relates to the study design and the quality control.

I am interested in knowing in these comparative matrix studies to what extent are the samples going to be analyzed blindly and simultaneously by the groups conducting the analysis?

DR. HUISINGH: We haven't addressed whether or not the samples will be conducted blindly; however, that could easily be done.

We are generally coding the samples when they leave our laboratory, and we have been saving an aliquot of the sample sufficient to do a salmonella bioassay. Dr. Bradow's group expects, I believe, to do some at least rough screening of the quantitating and profiling of the organics roughly about once a month over the time that those samples are being applied in the in vivo studies. Are they going to be done completely blind, Steve?

DR. NESNOW: They haven't been set up.

DR. HUISINGH: That could easily be done. Maybe that would be a worthwhile suggestion to make. Does that answer your question? The quality assurance is a difficult problem to address for a complex mixture in terms of sample integrity. We would welcome constructive suggestions in this area.

DR. SELTSER: I wanted to comment on that because it seems to me that the design that is presented in this and probably in subsequent studies does lend itself to a central group which could in fact code and distribute all samples so that there would be no questions later on.

It seems to me that there are certain things being phased in at different times, and I am concerned about the eventual interpretation of this data unless there is a fairly rigid mechanism of quality control in the blinding of samples to be done throughout this whole series of procedures.

DR. HUISINGH: I think that would be relatively easy to set up and we could certainly make sure that that happens.

DR. LEWIS: How many solvent systems have been tried other than methylene chloride and cyclohexane?

DR. HUISINGH: Roy, could you help me out? Larry Claxton, from our lab, has done these experiments with Dr. Zweidinger who is here.

DR. ZWEIDINGER: We tried about six different solvents. We tried cyclohexane, we tried cyclohexane-isopropanol azeotrope. We tried acetone, we tried direct sonification with DMSO.

DR. LEWIS: Which of these fractions was the most potent?

DR. ZWEIDINGER: The methylene chloride gave the higher--all the solvent extracts were normalized to the same volume and tested in the mutagenesis assay. The methylene chloride extract was found to be the most mutagenic.

DR. LEWIS: The second question I have deals with assessment of reproductive hazards. In the original design you showed when you talked about the paper in Williamsburg, that you assessed teratology for one thing. Did this assessment include integrity of chromosomes and chromatids and things of this nature?

In this study design I don't see the assessment of reproductive hazards from exposure to diesel exhaust emission as one of the biological response criteria.

DR. HUISINGH: I didn't get all of that. Did you refer to teratology studies?

DR. LEWIS: I am talking about reproductive hazards in toto, with teratology being one aspect of it, but also sperm abnormalities and things of this nature because this is something that is frequently overlooked by both toxicologists and people who are dealing with risk or hazard assessment.

DR. HUISINGH: We personally have no plans to do teratology studies. However, the Cincinnati protocols which will be reviewed later do include some reproductive studies.

Bill, will you be discussing that later? Do you want to defer that until later?

Bill Pepelko says that those studies will be described later when the whole animal inhalation studies are conducted. We have no plans today with limited funds and need to prioritize the program. We did not choose to recommend teratology studies on the organic extracts.

Do you feel that is needed?

DR. LEWIS: I am more interested perhaps in genetic damage to the reproductive system than to somatic cells.

DR. HUSINGH: Maybe I should say--I didn't point this out in the very first slide where I showed you the phased approach to screening. At the first phase where we are detecting activity and are using the salmonella assay to detect both potential mutagens and carcinogens, we are using the same assay for both endpoints. As we go up higher into mammalian cells and whole animal tests, we do, of course, separate these effects and we are looking either for a carcinogenic effect or the heritable mutation effect.

I didn't tell you about any studies we have done on whole organisms. We did conduct one drosophila test with the OXY fractions that was negative for sex-linked recessive lethal mutations. It is questionable, and anyone who would like to review that data could. There was dye put with the sample, and even though the dye did get into the fly, it is not clear that the mutagen got into the fly because they were not injected.

We have, because of resource limitations, and partly the needs of the regulatory part of the Agency, really been focusing on the carcinogenesis endpoint and have not produced a focus on really asking the question, do these materials cause heritable mutations?

That is something that is definitely lacking in the program and purposely so. I think at this time we did not foresee that there were adequate funds or rationale to go after that endpoint, and so that is really lacking, I think, in the whole program.

DR. LEWIS: We can discuss this in greater detail because it is such a broad subject matter, but I do want to emphasize that the testing of toxic potential of a chemical is different from tests that are applied in hazard or risk assessments, and I do want you to bear this in mind, that what you are looking for is those things that are most relatable scientifically to the assessment of hazard; that is, what is the risk under its intended use, rather that what is the inherent toxicity of a fraction of material of this nature?

DR. FLAMM: As was said several times earlier, the entire purpose of the exercise was to get data that will help better determine what the risk is to the public health, and I am sure that the people who have planned this work are constantly reminding themselves of that as well. The panel will have to do likewise tomorrow when they write their report.

I'm not sure that I have a full understanding of everything that Joellen said, but I would like to ask one question, and that concerns if, in this matrix where you have several different test systems, and you are looking at several different known carcinogens or several different types of exposures, some of which have some human epidemiologic definition, and others do not, but in this matrix approach, what would your attitude be if in fact you found that for test A you had one configuration of data, or one profile across the different substances or types of exposures, whereas with test B, C, and D you had entirely different ones. In other words, you could see no consistent pattern. What would your thinking at that point be, what would you make of that data, and how would you attempt to proceed to try to find a common denominator and explanation for that kind of variation?

DR. HUISINGH: Well, first of all, I guess we are making one basic assumption since the highest test, at least for sure planned, is the rodent skin painting test that will be conducted, as Steve will describe, in such a way that we will be able to look at initiation separated from promotion, as well as complete carcinogenesis.

If we make the assumption, and I'm not really sure that it is possible to do so with respect to extrapolation to humans, but if we make the assumption that at least the in vivo tests are more relevant than in vitro tests, than I guess we would, first of all, look and see how each one of the in vitro tests compared to the in vivo tests, and of course we are very interested in how those in vitro tests compare to each other, both in terms of sensitivity of detection, as well as concentration at which we get an effect of what is the scope of that line. Dr. Albert, would you like to make a comment?

DR. ALBERT: Yes. This is the sort of situation that has come up many times in the carcinogenic risk assessment business; for example, where one has a series of pathologists reading a slide differently.

We handle this by factoring it in terms of uncertainty. These differences in results give us a range of estimated risk. So I would think that the answer to the question is that if one gets a series of different potencies, depending on the measures that are used, and I expect that there will be because it is not only for different solvents or different mutagenesis tests, but mutagenesis versus skin painting versus inhalation, and so on.

I think all these results will give us a set of comparisons that cover a range, and the risk assessment will be described in terms of the range of comparative results.

DR. HUISINGH: I should also tell you that we have looked to a limited extent at the coke oven emissions and there are some things that are known about the chemistry of these emissions and that they are produced under reducing conditions rather than oxidizing conditions, and there is no reason to believe that the active material in any of these comparative sources is going to be the same, as all of them will contain probably more than one active component.

If they contain mutagens and if those mutagens are initiators, they probably, like the diesel exhaust, will contain compounds, such as phenols, that would be promotors; and since, for example, the oncogenic transformation assay presumably would take into account the effect you see would be more related to carcinogenesis, and it is known that you can pick up the effects of promotors as well as initiators in this assay.

The salmonella assay, for example, would only be detecting primarily the material directly interacting with DNA.

So I think we would agree with Roy: we would expect to see some differences, and at least so far in our preliminary data we do see some differences between the salmonella assay and the potency in that assay with different fractions compared to oncogenic transformation.

DR. FLAMM: I think one of the things that the panel might be able to contribute some suggestions to is with regard to how one can equate one test to another or find a common denominator, but I am beginning to express some of my own biases here with what's happening.

I wonder whether we could at this point break for coffee and try to come back in about 15 minutes. I would like, if we could, to have Joellen back for maybe another 5 or 10 minutes to see if we have any further questions before proceeding with the next presentation.

(A short recess was taken.)

DR. CORTESI: Before we get going again, I would like to ask, if possible, that we keep in mind, both in the presentations by the researchers and in the questioning by the panel on the issues that we hope that the panel will focus on, is the question of assessment of risk rather than the more researchy points of interest, both of the researchers themselves and the questions that one might have on that area really is how many people are we gassing out there and how seriously, is what we've got to focus on.

DR. FLAMM: Errol, I believe you had a question of Joellen?

DR. ZEIGER: I have a couple of points I would like to ask about that I felt weren't very clear. You described a number of tests, the Ames Test, mammalian cells in vitro for a number of endpoints. Are these various mutagenesis tests in vitro being done on all samples, or is the Ames Test being used to indicate which samples could be used for further testing?

DR. HUISINGH: The samples we described as the source comparison matrix--that was the second matrix I showed which includes the coke ovens, roofing tar, cigarette smoke--we plan to do the whole battery of tests, as I indicated, where the X's were, and those assays would probably--they could be initiated simultaneously and probably will be initiated simultaneously.

There are some potential problems in that some complex mixtures are difficult to test in some of these assays if they turn out to be too toxic, and we just don't know yet to what extent we're going to run into those problems.

DR. ZEIGER: A further question on that. I am assuming that if you get a positive in the in vitro tests, then those samples or those fractions will be further tested in the in vivo tests--skin painting, intratracheal, or other in vivo tests.

DR. HUISINGH: Not quite. Generally when we evaluate an unknown, like we did diesel, we use the phase approach that I showed. We do a salmonella assay, and based on that we will go on and do confirmatory assays, and then in vivo assays.

In this particular program involving the source comparisons, it has been planned that all of these assays on those critical sources that were identified in the matrix, and I think Dr. Nesnow is going to show that matrix again if you want to look at it again, all of those assays will be done, regardless of the results.

I can't really say they will all be initiated simultaneously because the skin painting tests have to be phased in depending on animal availability, but as much as possible, all these samples, as much as we can, will be evaluated in concurrent experiments wherever we can do that, and simultaneously, which we think will help eliminate some of the quality control problems.

DR. FLAMM: Yes, please?

DR. JONES: Since we have been admonished to review this program relative to risk assessment, I am extremely concerned about the applicability of information based on results using extracts to the real-life problem of inhaled diesel emission particles. It would seem to me that the results that one might get in the tests might be highly dependent upon the types of solvents that are used. In assessing the relative mutagenicity or carcinogenicity of roofing tar, cigarette smoke condensate, coke oven emissions and diesel extracts, one could, in fact, predetermine the results based on the selection of solvent systems used to prepare the extracts.

I would like to know what you are doing in the area of long-term dissolution with physiological solutions because I think in fact it may be that the effect dose is going to depend upon the solubility of these condensates on the particles in the physiological solutions.

DR. HUISINGH: That is very true. Our plan to date is to extract all of these materials, roofing tar, coke oven, both of those two with the same solvent, methylene chloride, and the cigarette smoke condensate, of course, will be tested as it is.

That could be discussed and possibly modified, and maybe Dr. Albert would have some suggestions on that. The exposure estimates involve some use of BSO's, benzene soluble organics, and BaP concentration, and it is not at all clear that because dichloromethane removes most of the mutagens or more of the mutagens than any other solvent tested, that that same thing would be true for either the coke oven or the roofing tar.

So, naturally, you can see that there is a lot of guessing going on here and we are trying to do the best we can. I think Dr. Bradow made one thing very clear, and maybe he would like to comment on it. He said that he is looking for order of magnitude differences, not little teeny differences, and to the extent that we can compare with what studies we have done with complex mixtures, and I don't know if maybe Dr. Epler has more experience with this and could make comments, but the order of magnitude differences that we see with different solvents is really within one order of magnitude.

I would have to go back and look at the data, but I don't think we see more than tenfold--well, that's probably not true when you are comparing to physiological solutions extractions where you may not get very much activity at all, but most of the organic solvents, the dose response region in the salmonella assay is all on the same horizontal axis, and we were able to get dose-responses within that axis, so we do see different slopes on those lines.

As far as physiological extractions, we think that is very relevant and some interesting work with fly ash and serum has been done and reported in Science, and we are doing similar work in how relevant serum is, or some lung surfactants.

It is really a research problem. It is not a very applied problem that we can necessarily say that we're going to do X, Y, and Z and get such-and-such a result by March. This program from the researcher's point of view, from my point of view, is frought with the need for a lot of applied work and maybe support from those of you on the panel that we need to do a little bit more research which would be very welcome from those of us back in the research end.

We are going to look at in vitro exposure of macrophages to these particles and see if we can detect release of mutagenic metabolytes, but even that, generally we can't do those studies in more than 24 or 48 hours, and presumably if these particles are down in the lung and are engulfed by macrophages or other cells and remain in the lungs for long periods of time, they remain there much longer than 24 to 48 hours. So I don't know how well we could ever mimic that situation, and I think that's why we need these in vivo studies where we look at some body fluids in these other assays to the extent that we can.

DR. JONES: I agree with you and that is why I raised the question. I think that the in vivo studies are certainly essential. My concern is that risk assessment is really an iterative process. Six months from now you are going to have the results of the in vitro studies and very little else for the first risk assessment iteration. I would therefore recommend a conservative use of these data in predicting risk to human health.

But at least in terms of the biological endpoint, it seems to me at that point you're not going to have too much else to go by, and I would caution one to be a little conservative at that point in time.

DR. ALBERT: The words that Dr. Jones used have a slightly sinister connotation. I would like to emphasize that the risk assessment process is strictly separated from the regulatory decisionmaking process.

The regulatory decisionmaking process has to take into consideration not only the risks, but an awful lot of other socioeconomic impacts and so on. I am sure you didn't mean that.

DR. JONES: I didn't mean that, Roy. I said one could, not would.

DR. ALBERT: Because this is going on the record--of course nobody is going to read the record, but nevertheless...

The other point was that I think it would certainly be useful to take a look at the comparative potencies using a few different solvents to see whether it makes all that much difference.

In terms of the kinds of evidence that we are going to have to present to the decisionmakers by the time they need it, which is next year--and incidentally that time frame is not set by the Agency, as I understand it--it is a time constraint imposed on it from outside.

The design of the research program deliberately took into account the extraordinarily short amount of time that we have to have to develop data. Certainly we hope that in addition to the in vitro stuff, that we have some useful skin painting data and also some useful strain A pulmonary adenoma data, so that there will be more than the in vitro, hopefully, by the time we are required to say something.

DR. FLAMM: Several of the panelists have indicated that they have comments or questions. Jim, Marvin, and Ray have all indicated that they have something to say, so let me begin with Jim Epler.

DR. EPLER: In line with the comments that Roy and Joellen have just made, in the closest aligned studies that have been carried out in which in vivo carcinogenesis tests have been compared with in vitro mutagenesis the results do not necessarily agree in terms of potential.

I guess what I am doing is extending another caution to you in this study to not rely completely, as you are not, on the in vitro type of testing.

A question for Dr. Huisingh, how do your results with the diesel fuel particulates compare with those that are already published concerning urban airborne particulates? Is it the same type of material that investigators are seeing in the urban particulates that you are seeing?

DR. HUISINGH: In general, yes, to the extent that the mutagenic activity from diesel particles is frameshift mutagen, it is direct acting, and most ambient air, at least that we looked at and that others have published on, has been primarily direct acting.

The nature of the mutagens in ambient air compared to diesel exhaust is a little bit difficult to comment on right now. We are in the middle of a project where we are fractionating ambient air particulate and looking at what fraction mutagenic activity is in.

There is activity in ambient air, as there is in the work you have done with synthetic fuels, in the basic fractions, and we did not see the major mutagenic activity in the diesel particle in the basic fraction, at least the way this fractionation was done. That might be one major difference, but I think we need to do more on ambient air before we can conclude on that.

Those results are significantly different, I might say, at least to this date, from what we have been able to look at in coke ovens. Coke oven emissions are primarily, at least so far, indirect acting, but the ones we have looked at were extracted with cyclohexane which is a more nonpolar solvent and that might account for the differences.

Also, the coke oven particulate extract was much more toxic and difficult to bioassay, so I don't really think any of us know yet what problems we are going to be facing.

There are methodological problems in some of the mammalian cell tests with these samples, and some of the tests, as I think were recently reported at a Department of Energy meeting, the cells come up off the dishes and do all kinds of things that you might not predict or don't happen with pure compounds, and it makes it impossible to carry out the tests in some cases.

Although the matrix looks good on the board, there may be holes in the end where we weren't able to adequately test the material.

DR. FLAMM: Dr. Goldman?

DR. GOLDMAN: It may be that I missed it, but I was not clear as to the extent of effort that is going into characterizing the particulates that you are dealing with. It is my understanding that there seems to be a general agreement, to date at least, that most of the biologic activity is surface-related to the extent that particle size distributions differ with the different engines you are testing and different fuel types you are testing. I am wondering whether a comparison based on just mass is going to be as correct as one that is perhaps more surface- or cubic-related.

DR. HUISINGH: The characterization part of the whole program is not even being reviewed here today. Most of that program, at least the research aspect of that program, is directed by Dr. Bradow, and I don't know whether it is appropriate from him to comment.

There is really a very major program on characterization. Ron, would you like to say something about that?

DR. BRADOW: With regard to the particle size distribution generated by diesel engines, I think the general evidence has been the particle size distribution does not vary with engine operating conditions significantly. All of the particles essentially are below 2 microns; at least the whole mass of the particle emissions was below 2 microns in almost every case I know of.

There are some very fine particles. There are some small shifts of particle size distribution in the fine particles with varying dilution ratio. However, even in those cases the bulk of the material in the particle size range, that is certainly 95 percent of the mass of particle emissions, is in the particle size range which is normally considered hazardous, that is respirable, in the 2 microns, at say, 0.05 micron range.

So with regard to particle size distribution, there seems to be nothing there to hang your hat on. In fact, we don't normally even bother to measure it.

I think one major issue is the ability of physiological fluids to extract the mutagen compounds. That we don't know very much about. I think of deposition of carbonaceous aerosols in lungs, is something that is not very well established. The clearance mechanism, the rate of clearance, also is not very well established, so it is very difficult to calculate a dose rate on the basis of an exposure.

I am not sure to what degree that really influences any decision-making, but at least from my point of view as a research scientist, I consider that a relatively large gap.

DR. FLAMM: I would certainly agree with you, and I think that is highly relevant to estimating human risk.

DR. GOLDMAN: If we're going to compare these median sizes around 2 micrometers in diameter to other sources, are we going to normalize in our risk assessment to 2 micron samples of coke oven emissions? In other words, I'm concerned that we not get into an apples versus peaches comparison in which the quality of the particles is so markedly different aerodynamically and with regard to its surface properties that a comparator based on mere mass proved to be misleading.

DR. HUISINGH: Some of you may not be familiar with the massive volume sampler that is going to be used to collect the coke oven sample. This sampler was designed originally to collect large samples for bioassay.

It has two impactor stages and a final precipitator stage. We are right now doing some shakedown experiments. The collector does, in the precipitator stage, generate very low amounts of ozone, about 0.02 parts per million which is comparable to some city air. It is possible, however, to operate that sampler in a less efficient mode without any ozone.

So we are doing some experiments right now to look at operating with or without ozone present. We will compare the mutagenic activity of samples collected under both conditions. We have already looked at them at the different stages of the impactor and the final electrostatic precipitator, and they are collected so that you can just look at the respirable particles.

That is what we are planning on doing, and that's why we are not looking at coke oven door seals. We are actually taking two ambient air samplers up on top of a coke oven, which was difficult to arrange, and collecting size-fractionated particles up there.

Those particles will be scraped off the electrostatic precipitator plates and handled as the diesel particles will be handled.

I don't know if that makes it a little clearer, but they will be the respirable particles that will be looked at.

DR. FLAMM: There will be a presentation on inhalation studies, and perhaps we can get back into this matter at that time.

DR. WILEY: We seem to have centered on this question of the solubility of carcinogens from particles. I agree with Ron Bradow that the particles are in the micron, submicron to one micron range, but I don't see within the in vitro studies where we are going to concentrate on trying to answer this question which is maybe the place where it can be answered best; that is, what is the risk relevant to the solubility of the carcinogens from the particles which are inhaled?

I wonder if we aren't at the point now where we are saying that greater emphasis should be placed upon an examination of the solubility of carcinogens from the particles in order to aid in the design of the in vitro studies.

DR. FLAMM: I think that's a very good point and it is one that others are making as well, but really there are sort of three parts to the problem in certain respects.

One is what are the general ambient exposures, and then how do you go from the exposure to really the dose, the physiological or toxicological dose, to the organism? And then, thirdly, what is the potency of the substances that make up the dose? I think perhaps tomorrow we may want to address those individual steps, even though I suppose certainly the work regarding exposure, particle size, characterization, or what not, is outside our purview. Nevertheless, this is a stepping stone to any effort that seeks to determine a risk to the public health.

I think that we're going to have to go on with some of the other talks, but we will certainly come back to this and again back to Joellen and others as we attempt to put our report together tomorrow. We will certainly get back to the very question that you are posing which clearly is a fundamental one as regards to what the human risk may be.

DR. SELTSER: I would like to get a little clearer on just what the potential problems are, the real problem in terms of using a gasoline exhaust as a possible comparison. If we're talking about risk assessment, in terms of what is eventually needed, you are talking about relative risk rather than absolute risk.

I think that Dr. Albert pointed out that there is no real problem in terms of defining whether or not there is carcinogenic material in exhaust. The question is in terms of the substance to which this is to be compared.

If the decision at the regulatory level is no diesel, it means more gasoline, so consequently it seems to me the critical feature would be a comparison between exhaust, gasoline exhaust and diesel exhaust, if that is possible.

Now you heard the problem about developing material. It is difficult to get gasoline exhaust material; it is easy to get diesel. That is almost like looking under the lamppost for the watch that you lost down the street, but it's easier to find it under the lamppost.

The question is how difficult is it? I would think that it would be useful to have information about a noncatalyst, lead-burning gasoline engine as well, if you couldn't get enough material from catalytic engines because I suspect that now and in the future a considerable amount of our air pollution is coming from automobiles which for the immediate future and for a considerable period of time will not in fact be catalytic or will perhaps not be safe enough to meet present standards.

I would like to really be clear on how impossible is it to incorporate the gasoline exhaust as one of the comparisons.

DR. FLAMM: I think the answer to your question can be broken down into various test systems that they are proposing to apply, so I think that the question relates to what can you do with the in vitro, and it relates again to the inhalation work and again to the skin painting.

I think that we're going to have to ask that question each time around and see what is possible since obviously that is a central question. Obviously the comparison does make a great deal of sense, and we're just going to have to look at the feasibility that you suggest.

DR. CORTESI: May I make one comment?

DR. FLAMM: Sure.

DR. CORTESI: The diesel cars, if they come in, will be replacing cars that presumably are meeting current standards for air pollution control. So in comparison to what--comparison to old cars--is not relevant because it is not a question of a new diesel versus an old Packard, say. It's a question of a new diesel versus a new car meeting air emissions standards.

DR. BRADOW: May I make some comments please, Dr. Flamm?

DR. FLAMM: Sure.

DR. BRADOW: I think there is a strong possibility that the controlling factors and the comparisons that are being discussed now are the mass emission rates, and consequently the human exposure to those materials to a greater degree possibly than the relative potency of those materials.

For example, the pilot runs which have been done so far in the Ames Test, only one automobile, catalyst automobile, was involved there. I believe that Joellen's reports back to us indicated that the potency of the gasoline engine catalyst emissions in that one case where a sample was obtained were roughly equivalent to those of the diesel, perhaps even a little on the high side of what was available from diesel.

However, the mass emission rate, in other words the total mass of material delivered in the ambient air, is about two orders of magnitude below that of diesel.

This was a particularly peculiar automobile in that some organic aerosols were present in the exhaust. In many cases with an automobile running--with basic engine operation with effectively operating catalysts, we are comparing something with nothing, and the matter of being able to rate the potency of a virtual zero emission may be pretty academic.

In the case of a catalyst of automobiles burning leaded gasoline, we certainly can acquire samples large enough to do some of the simple bioassays. However, the requirement of material for skin painting tests or for intratracheal instillation that I simply do not know how to get enough material to do that.

I think in the end the job that needs to be done here will be resource-limited. The question is where do you want to spend the resources, and if it is desired really to get a large amount of material from catalyst automobiles to run such tests as intratracheal instillations or skin paintings, then the existent test facilities for automobiles in the United States are not large enough; there are not sufficient number of them to be able to generate these materials.

We are talking now about a generation rate for the diesel case, in order to carry out the whole program, of about 12 kilograms of material. We are exhausting the available test facilities now to generate the diesel samples. When we talk about a sample from gasoline cars which emits a hundredfold less material, there is simply no way that we can do that. It is not possible.

DR. FLAMM: We said only if feasible.

DR. SELTSER: You mentioned the inhalation and skin paintings. What about the in vitro studies?

DR. HUISINGH: We're going to try to whatever extent we can, with Dr. Bradow's support of the emissions characterization part of the Agency that exists up in Ann Arbor, they have some ongoing programs where they will be collecting filters.

The way I understand the problem from a nonengineering point of view, when they change those filters they are white, and when they change the diesel particle filters they are black, and they can collect lots of white filters and extract them, but they don't have anything, and we barely have enough to barely do--if we can get enough organics to do the same dose range--we see biological activity in the same rough range on the couple of very limited samples we have been able to look at.

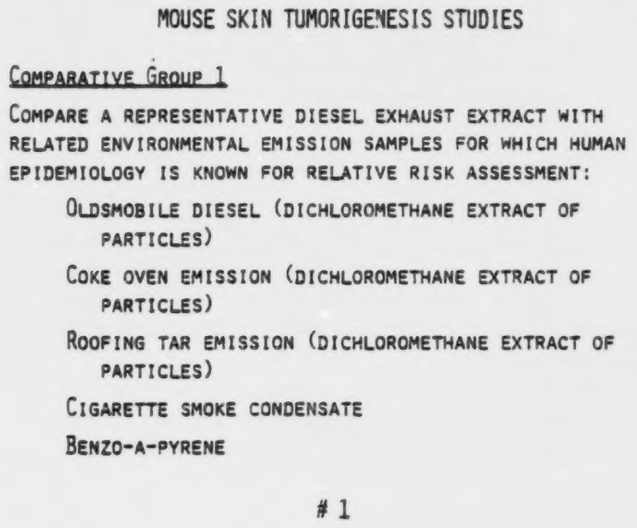
So far we have not had enough samples to do all five tester strains just in salmonella, and we are ready to do whatever anyone can send us samples on. If industry wants to send us samples, I'm sure we'll be glad to get them. Maybe I should take that back. It's really an engineering problem and it's not a biological problem at this point.

DR. FLAMM: I think at this point we need to go on to the next speaker, who will be talking to us about skin painting studies, Dr. Stephen Nesnow.

DR. NESNOW: Thank you, Dr. Flamm. I would like to spend some time here in going into a little more detail in the skin tumorigenesis studies which have been alluded to many times in the past hour.

I first would like to start out by describing the samples which we will test, then describe the specific details of the test system, and finally discuss some requirements and some of the outputs that we will be providing.

May I have the first slide, please? (Slide #1.)



MOUSE SKIN TUMORIGENESIS STUDIES

Comparative Group 1

Compare a representative diesel exhaust extract with related environmental emission samples for which human epidemiology is known for relative risk assessment:

- Oldsmobile diesel (dichloromethane extract of particles)
- Coke oven emission (dichloromethane extract of particles)
- Roofing tar emission (dichloromethane extract of particles)
- Cigarette smoke condensate
- Benzo-a-pyrene

1

The first slide is a description of the first comparative group of samples which Dr. Huisingh has already discussed which compare a representative diesel exhaust extract with related environmental emission samples.

As we have heard before, the samples we will be testing will be a coke emission sample, a roofing tar emission sample, and a cigarette smoke condensate. This will be compared to two standards. One will be the Oldsmobile diesel which we consider to be our representative diesel sample, while the other is the well-known air carcinogen, benzo-a-pyrene.

Just to reiterate, the top three samples on the list will be dichloromethane extracts. They will be the complete extract of the particles. The cigarette smoke condensate is not a particulate sample per se but a condensed emission product.

The next slide, please. (Slide #2.)

MOUSE SKIN TUMORIGENESIS STUDIES (continued)

Comparative Group 2

Compare a group of diesel exhaust extracts with different mutagenic responses in the Ames Test across a battery of short-term and whole animal bioassays:

Oldsmobile diesel
Nissan diesel
Turbo-Rabbit diesel
Caterpillar 3304 diesel
Mercedes diesel
Benzo-a-pyrene

This slide describes the second comparative group of samples which compare diesel exhaust extracts, each possessing different mutagenic responses in the Ames Test across a battery of short-term and whole-animal bioassays, and this bioassay is obviously the mouse skin tumorigenesis model.

These are the samples we plan to bioassay and hope to obtain all of them; Oldsmobile, Nissan, Turbo-Rabbit, Caterpillar, and Mercedes, and compare them to benzo-a-pyrene.

May I have the next slide? (Slide #3.)

We intend using the mouse skin tumorigenesis model to examine four different phenomena which I will describe in detail in the next couple of slides. We will examine the ability of each of these materials to act as tumor initiators, tumor promotors, cocarcinogens, and as complete carcinogens.

MOUSE SKIN TUMORIGENESIS STUDIES (CONTINUED)

SUMMARY OF PARAMETERS

PROTOCOLS: TUMOR INITIATION
TUMOR PROMOTION
COCARCINOGENESIS
COMPLETE CARCINOGENESIS

DOSES/PROTOCOL: 5

ANIMALS/DOSE: 40 MALES AND 40 FEMALES

MOUSE STRAINS: SENCAR
C57 BLACK

3

We plan that for each protocol or phenomenon we will test with five doses so that we can hopefully obtain a dose response. For each dose we will be employing 80 animals--40 males and 40 females.

Further, we propose to use two mouse strains, the Sencar mouse, which is an acronym for a mouse strain bred for sensitivity to the carcinogenic effects of polycyclic aromatic hydrocarbons, and the standard C57 black mouse strain.

Now, the Sencar mouse was developed by Dr. Boutwell at McArdle Laboratory for Cancer Research and Dr. Thomas Slaga at Oak Ridge National Laboratory. These studies will be carried out at Oak Ridge under the direction of Dr. Slaga.

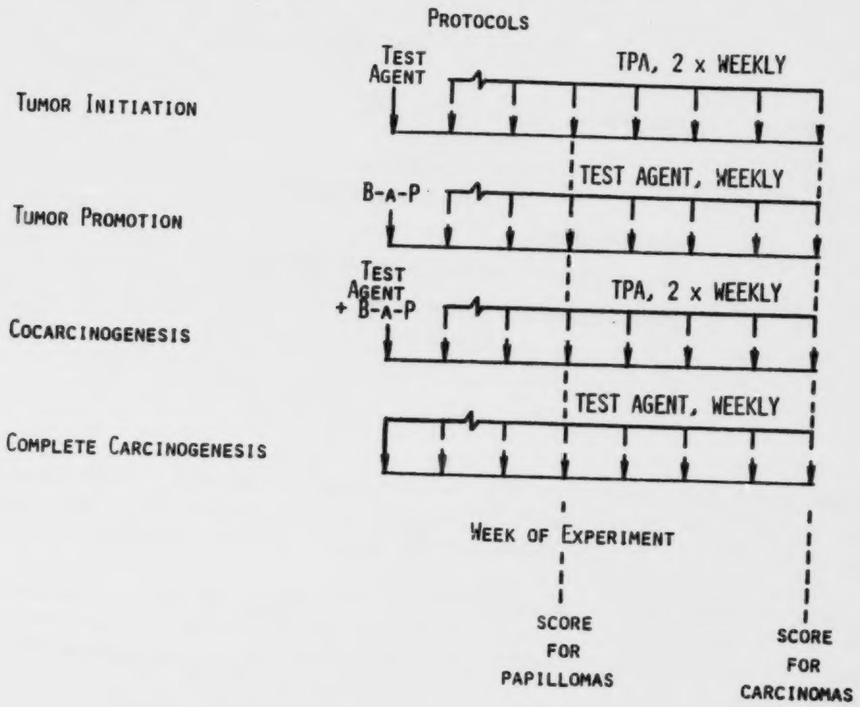
If we add up numbers, the total numbers of animals we are talking about in these experiments comes to approximately 24,000. (Slide #4).

These are the individual protocols which describe the different parameters which we will measure.

Tumor initiation is defined as applying the test agent once or several times in a very short time period, waiting a week, and then applying a tumor promotor once or twice a week for an extended time. The promotor we will use is TPA, a very potent tumor promotor. TPA, or tetradecanoylphorbol acetate, is a dierpere ester isolated from chroton oil.

For tumor promotion we give a standard tumor initiator, in this case BaP, benzo-a-pyrene. We follow that with application of the test agent, applied at weekly intervals, possibly up to 60 weeks.

MOUSE SKIN TUMORIGENESIS BIOASSAYS



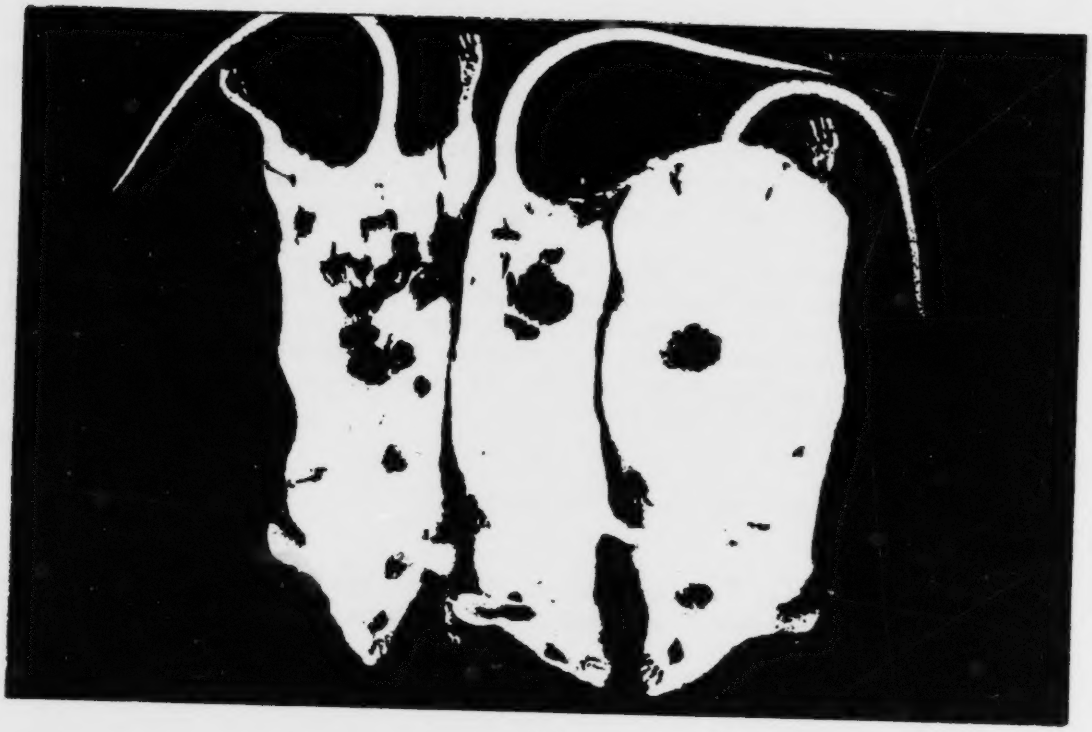
4

Cocarcinogenesis will measure the effect of these exhaust extracts on the carcinogenic effect of BaP. There are two ways of performing this. The way I have described, in which the test agent and BaP are applied once simultaneously. We could really reterm that coinitiation. After this treatment, the animals are treated with the promotor twice weekly. The other alternative is to apply it with the carcinogen at multiple times across the whole 60-week experiment, but due to sample limitations, we will not be able to do that.

Complete carcinogenesis is where we repeatedly apply the test sample once weekly for 60 weeks.

In all of these protocols we will score for papilloma formation which, as some of you know, is a benign tumor. In two of these protocols, we intend to carry the experiments for 60 weeks to score for the formation of malignant carcinoma in addition to the papillomas. That would be the tumor initiation and the complete carcinogenesis protocols.

Dr. Cortesi once mentioned he wanted to see what a papilloma was. (Slide #5.)



5

These are three mice. The one on the left has multiple papilloma formations on it. I have a blowup of this on the next slide. (Slide #6.)

6

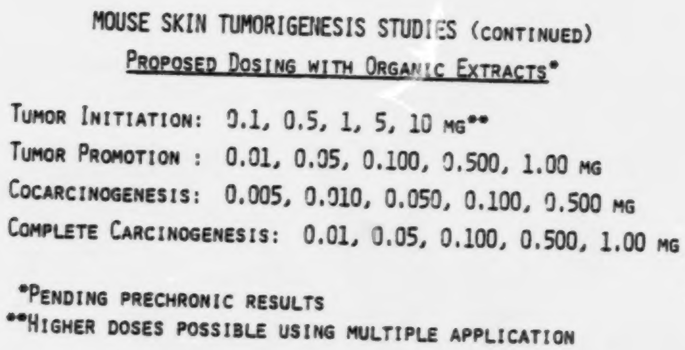
The one on the left has multiple papillomas. Those are the small dark nodules. The one in the middle has both papillomas and carcinomas. The animal on the right has the carcinoma. These are the kinds of tumors that you observe. With the very potent carcinogens like benzo-a-pyrene or 7-12 dimethylbenz(a)anthracene you get multiple papillomas.

There have been kinetic studies performed, some of which were reported by Dr. Albert, that show a portion of the papillomas are converted into carcinomas, and so the overall papilloma yield is a good indicator of carcinoma formation. Also, in complete carcinogenesis experiments we observe few papillomas while with the tumor initiation experiment we see large numbers of papillomas.

DR. FLAMM: Don't you shave the backs?

DR. NESNOW: Yes, they are shaved, but the hair grows back and we shave them again until we reach a point where the tumors interfere.

May I have the next slide. (Slide #7.)



MOUSE SKIN TUMORIGENESIS STUDIES (continued)

Proposed Dosing with Organic Extracts*

Tumor Initiation: 0.1, 0.5, 1, 5, 10 mg**
Tumor Promotion : 0.01, 0.05, 0.100, 0.500, 1.00 mg
Cocarcinogenesis: 0.005, 0.010, 0.050, 0.100, 0.500 mg
Complete Carcinogenesis: 0.01, 0.05, 0.100, 0.500, 1.00 mg

*Pending prechronic results
**Higher doses possible using multiple application

7

These are the doses that we propose to start with; pending our pre-chronic studies. Every agent which we will test will be evaluated initially for toxicity. With the tumor initiation experiment, we will also have a 50 milligram sample, if that is possible, doing five multiple 10-milligram applications. This is really pushing the system, but since we have no idea right now of how potent the samples are, we have to sort of cover all bases here.

The tumor promotion experiments will be carried out in a dose-response manner. The high dose is limited since it is applied at least 20 times for a papilloma formation. This dose is limited to the toxicity that these mixtures will elicit.

Cocarcinogenesis is one application so we can go to a higher dose applied while complete carcinogenesis, like promotion, is limited to the number of times that you apply it to the backs of these animals. We estimate that 1 mg/animal/week will be the maximal amount we can apply.

The next slide, please. (Slide #8.)

MOUSE SKIN TUMORIGENESIS STUDIES (CONTINUED)
DOSE RESPONSE CONTROLS (EACH STRAIN - EACH SEX)

TUMOR INITIATION:

BENZO-A-PYRENE (B-A-P)	TETRADECANOYLPHORBOL ACETATE (TPA)
0.25, 1.2, 2.5, 12.6, 25.2, 50.4, 100.8 µG	2 µG, 2 X WEEK

TUMOR PROMOTION:

B-A-P	TPA
50.4 µG	0.1, 0.5, 1, 2, 4 µG 2 X WEEK

COCARCINOGENESIS:

B-A-P	PYRENE	TPA
25.2 µG	10, 25, 50, 100, 200 µG	2 µG, 2 X WEEK

COMPLETE CARCINOGENESIS:

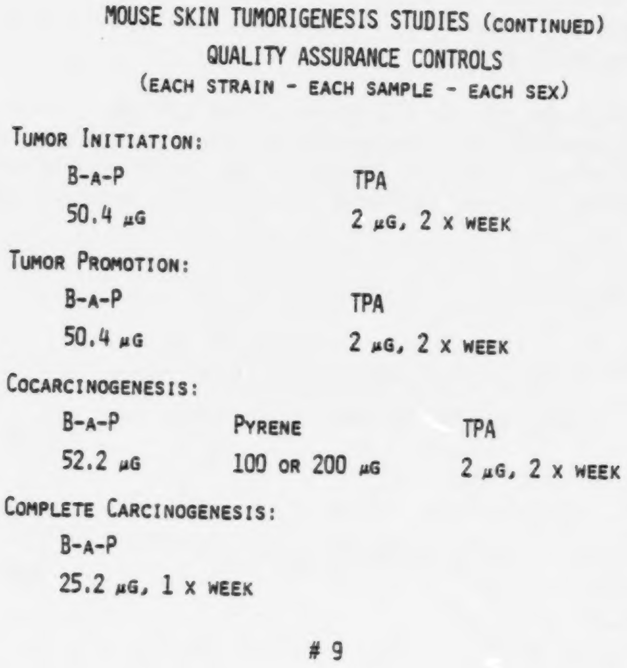
B-A-P

1.2, 2.5, 12.6, 25.2, 50.4 µG
1 X WEEK

8

The next slide describes some of the controls which we plan to incorporate into this overall program. The first set of controls, which I have called dose-response controls, has to do with comparing these agents with the results which we will obtain with the emission samples. Each is going to be done in a dose-response manner and each will be done for each strain and each sex.

The next slide. (Slide #9.)



MOUSE SKIN TUMORIGENESIS STUDIES (CONTINUED)
QUALITY ASSURANCE CONTROLS
(EACH STRAIN - EACH SAMPLE - EACH SEX)

Tumor Initiation:

B-A-P	TPA
50.4 μg	2 μg, 2 x week

Tumor Promotion:

B-A-P	TPA
50.4 μg	2 μg, 2 x week

Cocarcinogenesis:

B-A-P	Pyrene	TPA
52.2 μg	100 or 200 μg	2 μg, 2 x week

Complete Carcinogenesis:

B-A-P
25.2 μg, 1 x week

9

This slide shows the quality assurance controls. We want to make sure that a test in January is comparable to a sample that we are going to be testing in June. Each time a sample is bioassayed, there will be a set of animals treated at one dose for tumor initiation; with BaP as the initiator and TPA as the promotor; for tumor promotion; with BaP as the initiator, and TPA as the promotor and so on. This will be done for each sample and each strain and sex to normalize variations.

Tne next slide, please. (Slide #10.)

The next slide gives you an idea of how much sample will be required. This is not particulates, but is extracts. The slide summarizes the sample requirements for each protocol assuming a maximum MTD of either 1, 1.5, 2, or 2.5 milligrams per animal per week. In fact, if we just wanted to test for tumor initiators, we could bioassay the automobile emissions. Promotion and complete carcinogenesis require the majority of the total sample.

We need for each strain, based on an MTD of 1 milligram per animal per week, approximately 20 grams. Adding the prechronic requirement and for two strains, we would require about 40 grams of extract to test each sample.

SKIN CARCINOGENESIS

	SAMPLE REQUIRED (G) BASED ON MTD OF:				
REQUIREMENTS FOR CHRONIC EXPERIMENTS (ONE STRAIN)	1.0	1.5	2.0	2.5	(MG/ ANIMAL/ WEEK)
INITIATION + COCARCINOGENESIS	0.32	0.48	0.64	0.80	
PROMOTION + COMPLETE CARCINOGENESIS	19.2	28.8	38.4	48.0	
TOTAL	19.54	29.38	39.04	48.8	
PRECHRONIC REQUIREMENT (ONE STRAIN)	0.25	0.25	0.25	0.25	
TOTAL (ONE STRAIN)	19.79	29.53	39.29	50.05	
GRAND TOTAL (TWO STRAINS)	38.58	59.06	78.58	100.1	

10

May I have the next slide, please? (Slide #11.)

This is the milestone schedule which I hope we can stick to. The salient point here is that by August or September, we will have the papilloma data for the first four samples.

These first four samples will be our matrix studies comparing the Oldsmobile diesel with coke oven, roofing tar, cigarette smoke and BaP. We will have papilloma data by that time period, and as the experiments continue, we will have carcinoma data also. All of the samples will be complete by September 1980.

In addition to quality control for biological variation, we plan to quality control the individual test samples. We will do that by testing each sample monthly in the Ames test.

Right now no decision has been made as to what would happen if we found a sample losing activity or to what level a new sample should be generated. Of course, we are hoping that these samples are stable through the duration of this program. That is basically what I have to present, and I will be glad to answer any questions you might have.

SKIN CARCINOGENESIS
MILESTONE SCHEDULE

Date Yr/Mo		Increase Breeding Stock	Sample Number (both species)						
			1	2	3	4	5	6	7
1978	Nov	Start							
	Dec	Comp.							
1979	Jan		Start						
	Feb		X	Start					
	Mar		X	X	Start				
	Apr		X	X	X	Start			
	May		X	X	X	X	Start		
	June		X	X	X	X	X	Start	
	July		RP	X	X	X	X	X	Start
	Aug		X	RP	X	X	X	X	X
	Sept		X	X	RP	X	X	X	X
	Oct		X	X	X	RP	X	X	X
	Nov		X	X	X	X	RP	X	X
	Dec		X	X	X	X	X	RP	X
1980	Jan		X	X	X	X	X	X	RP
	Feb		X	X	X	X	X	X	X
	Mar		RC	X	X	X	X	X	X
	Apr			RC	X	X	X	X	X
	May				RC	X	X	X	X
	June					RC	X	X	X
	July						RC	X	X
	Aug							RC	X
	Sept								RC

RP - Report score on papilloma formation
RC - Report score on carcinoma formation

11

DR. FLAMM: Let me ask the panel whether they have any questions at this time for Steve.

DR. EPLER: Steve, if the materials are potent, the time frame that you have established is probably adequate. But if they are low level, it is probably not. How are you going to cope with that and still conform with your deadlines?

DR. NESNOW: You can't push nature beyond certain limitations, and if tumors don't arise by our deadlines, then we will not have that information. Outside of doing the things that we propose, in terms of dosing, I don't see any way of obtaining this information if we don't see tumors within the first 20 weeks.

DR. ZEIGER: This might be a little bit out of my major area, but is anything really known about the correlation between potency in skin painting experiments and potency in inhalation experiments, which is, I gather, what will be the main route of exposure to these particulates. If we do get carcinogenesis with some of these extracts, and I think I would be surprised if we didn't get carcinogenesis, what predictions can you make based on the human route of exposure, which will be inhalation?

DR. NESNOW: Well, we intend to get the inhalation data, and the laboratory at HERL/Cincinnati will describe this later today. What we are faced with right now is not picking the best system. What we are faced with is getting as much data by August as we possibly can, and then sifting through it and finding out what correlates and what doesn't. The mouse skin tumor model was chosen because it is one of the few bioassay systems which will give you in vivo tumorigenesis data within 20 weeks. In terms of correlation, the papilloma data correlates very well with the resulting carcinoma data.

I would also like to point out that inhalation may be the main route of exposure. But it's not the only route of exposure. We are also being exposed dermally as well as orally. The routes of exposure and their relative hazards have yet to be determined.

Your question is well taken, and will probably be answered later on when we evaluate what kinds of bioassays can be used for evaluating crude effluents and particulates.

DR. ALBERT: There isn't much basis for comparing the comparative potencies by assay in mouse skin and by inhalation. There are a few instances with direct acting alkylating agents like bischloromethyl ether, epichlorohydrin, and dimethylcarbomoyl chloride.

When one looks at the comparative potency as inducers of skin tumors and with the induction of cancers of the respiratory tract, which all three agents incidentally do and have been tested, and they have been tested both ways, the correlation is not so bad. But there is a very limited degree of correlation that can be established, because not all that large a number of agents have been tested by inhalation that would permit the comparison to be made.

DR. ZEIGER: I think we are back here to the initial questions before Dr. Nesnow's talk on dosimetry. You know how much of the total extract is getting onto the skin. There is no information now, and I don't know if any is planned, on seeing how much of the total organics are administered to the animal by inhalation.

Now I'm talking about the number of particles, but the actual elution of those particles in the lungs. And there we might be talking about a completely different class of substances than those that are active on skin painting, because you are not only looking at polycyclic aromatic hydrocarbons, you're looking at aromatic amines, and I gather nitro compounds as well.

So, I was just wondering if this massive amount of effort here on skin painting might not be better directed toward getting some of the dosimetry by the route of the human exposure, and not looking at this skin painting, where essentially you know the outcome. It is just a question of how many papillomas you're going to get, how many carcinomas.

DR. FLAMM: Dr. Jones?

DR. JONES: I was just going to make the comment that I think probably the correlation between skin painting and intratracheal instillation of the extract may be quite good, but I think the correlation between the skin painting of the extract and inhalation of the raw diesel is going to be really not comparable, so I think that therein really lies the rub.

Once again I come back to the point that risk assessment is an iterative process. The first thing that you're going to have is the in vitro test results, the next thing you're going to have is the skin painting results, again using the extract, but for the humans out there in diesel land, the important dose is the dose that results from the inhalation of the particles.

DR. NESNOW: I agree with your comments. I would like to say that we have an overall diesel carcinogenesis program here. What you have heard is the first two parts of it. We do plan to expose animals to whole exhausts.

There is no way you can get that information by August, but we will have those experiments in progress, and we will eventually have all of this information. For the short-term we are doing what we think will provide the best and most credible information.

DR. ALBERT: I wonder whether or not we're not making a little too much out of the notion of dosimetry because you are dealing with complex mixtures, and the question is dose of what? I think it depends on how the data comes out. I think that, for example, if it turns out that all these preparations show roughly the same potency, comparative potency, with the variety of tests that are being used, whether it be in vitro assay, mutagenesis, oncogenic transformation, or skin painting, and so on, that this will make life simpler, or comparatively simpler.

But on the other hand, if all the tests go in all different directions in terms of the kinds of comparative effects that one gets, it will make life comparatively miserable.

So it's a little difficult to know how this is going to turn out. It seems to me that we are dealing with the practical issue of generating some approach that seems reasonable without any pretense that there aren't substantial flaws in the approach.

DR. FLAMM: Well, it's good to know that optimism isn't dead.

DR. NESNOW: I would just like to make one further comment, and that is we are also going to look for tumors at other sites in the mice. In other words, we are going to, at the end of some of the 60-week experiments, take the animals treated at the highest dose and perform histological analysis on a variety of tissues, not only skin, but other organs and other sites. We will see whether the extract acted at nondermal sites.

DR. FLAMM: Steve, is the solvent system that you are painting to the mouse's back methylene chloride or dichloromethane?

DR. NESNOW: No. Actually none of the solvents--dichloromethane is the solvent that was used to extract the organic materials off the particles, but actually in terms of solvents applying organic materials to bioassays, there are a number of them. The skin system can use acetone or DMSO. The Ames Test currently uses DMSO. As you know, most of the systems are aqueous and these materials are organic.

DR. FLAMM: Which ones do you plan to use?

DR. NESNOW: We would prefer to use acetone.

DR. FLAMM: That is the one that is typically used.

DR. NESNOW: We prefer to use acetone and for the oncogenic transformation work we are also using acetone.

DR. FLAMM: I just meant for the skin painting. Are you taking any special precautions with regard to visible light, white light?

DR. NESNOW: The precautions that we are taking are as follows. Each sample is being applied to the mice under yellow lights. However, the mice are housed in rooms with standard fluorescent lights. I think Dr. Epler has also had experience with this.

DR. EPLER: All of the studies that we have carried out with synthetic fuels have been carried out under yellow lights. The skin painting application carried out by Dr. Holland's group is also carried out under yellow lights. The animals, however, are housed, at least for some portion of their lives, under normal light conditions.

DR. FLAMM: Getting back to the central theme or bottom line regarding risk to public health, basically the skin painting is hoped to provide some comparative information as regards to the strength of carcinogenicity of the extracted diesel particulate materials compared to cigarette smoke condensates. You indicated that perhaps in this instance because only X number of grams were required for the skin painting study, that it might indeed be possible to use the catalytic emissions. Isn't that correct?

DR. NESNOW: I think if we could come up with about a half a gram of sample, we could test it in one strain. I don't know how difficult it is to come up with a half a gram of sample.

DR. BRADOW: Possible, but tough.

DR. FLAMM: That's better than impossible. Are there any further questions of Steve by the panel?

DR. WILEY: Just a comment on the timing. I have just read a study here which was conducted by two toxicology labs in Hamburg and Heidelberg, Germany, in which they painted on condensates from a Volkswagen engine, and the earliest tumor incidence that I can find here is about 3.2 percent--this is a skin painting study--at about 52 weeks. So if there is a comparable potency here, 20 weeks may just be a bit too optimistic.

DR. FLAMM: How frequently was it painted?

DR. WILEY: Three times a week, and roughly in the same concentration that you were describing.

DR. NESNOW: I gather that you're talking about complete carcinogenesis and not tumor initiation.

DR. WILEY: That's correct.

DR. NESNOW: That would, of course, take about 50 or 60 weeks. In terms of the tumor incidence, it's hard to know what to expect. With the very strong carcinogens we can see up to 20 papillomas per mouse, and obviously if we don't see tumors at 20 weeks, we'll just keep the animals. We're not going to sacrifice animals until we get the data. If it happens to go beyond August, then that's just the way it's going to be.

DR. SELTSER: I just wondered how large a difference between samples would you be able to detect in a study this size?

DR. NESNOW: We are looking at 40 animals per group, males and females, and I imagine under certain conditions we would combine them. So we will be looking at maximal range of about 20 papillomas per mouse, and a minimum range of 1 in 80, or somewhere in between there.

Would you agree with that, Dr. Albert?

DR. ALBERT: Yes.

DR. FLAMM: At least two logs, and then you might have a 2 or 3 log difference in terms of the mass that you are applying, particularly if you are dealing with something that is known to be highly carcinogenic and you would be applying it in a very dilute solution, as with benzo-a-pyrene.

DR. NESNOW: Of course, we are doing a complete dose response for each agent under each condition, and we hope to be able to define both the noneffective doses as well as the highest maximum dose. Again, it is a matter of having to start a program without having to spend much time in doing preliminary work in order to home in on the optimal conditions.

DR. FLAMM: I noticed you are not using DMBA, dimethylbenzanthracene, and I was wondering why since there is a very good data base on it.

DR. NESNOW: Yes, there is a very good data base for DMBA. We chose BaP because in some of the epidemiological studies that Dr. Albert has referred to, the coke oven and the roofing tar, they actually measure BaP as an indicator, so we have exposure, information and, I guess, we would also have BaP contamination in the diesel samples also.

DR. FLAMM: I wouldn't for the world criticize your use of benzo-a-pyrene for these studies. It is clear that you need to have that. I just wondered whether it wouldn't be useful to have DMBA as well in case certain questions were to arise. You could perhaps usefully tie your experience into the rather large data base that used DMBA sometimes just as a positive control.

DR. NESNOW: I'm sure that we could. It wouldn't be too difficult.

DR. FLAMM: Further questions, panel?

(No response.)

Thanks very much, Steve. I am sure we will be getting back to you again tomorrow as we struggle with our report outline.

We will go on to the next speaker who is William Pepelko who will be talking to us about intraperitoneal injection studies.

DR. CAMPBELL: I'm Dr. Campbell. I wonder if we could maybe make a little adjustment here. In our guidelines for preparing for this, I was going to cover whatever injection studies we were doing at Cincinnati along with other material, and Dr. Pepelko was going to cover some different material entirely.

The injection study slides that I have are in among all others and not in the machine, so if there is any adjustment that could be made, I would suggest that we do it at this point.

DR. FLAMM: I don't see that there is any problem at all, Dr. Campbell; whatever you propose. Did you want to go ahead and give your talk at this point?

DR. CAMPBELL: I was going to suggest lunch because I don't have any material loaded in the machine. The only other suggestion I might make is that the other work that is being done that fits in between the work we've heard of and the inhalation work might come in at this point, but I believe that would be split up by lunch, so that might be a little difficult, and I don't think they are in the machine yet either.

DR. FLAMM: I don't think it would do any great violence to us at all if we were to take up at this time the intratracheal instillations and injection studies.

DR. CAMPBELL: He has the same problem. They're not in the machine.

DR. ALBERT: Could I raise an issue about condensates?

DR. FLAMM: Yes. Let's come back to order, please. Dr. Albert wants to raise an issue vital to these deliberations.

DR. ALBERT: Since there is nothing better to do, I thought that I would raise the issue that I think is still somewhat unresolved, and that is whether in addition to the studies that are being proposed, there ought to be a concerted attempt to look at condensates as well as particulates.

To my mind this really hasn't been answered. Is Dr. Bradow around? I think sooner or later this question is going to come up, you know. The program has looked at particulates, but what about those condensables? Is there anybody that can talk to that in terms of--

DR. ZWEIDINGER: Are you referring to the gas phase materials that go through a filter that you would take particulates on?

DR. ALBERT: Yes, particularly those things that are going to condense, that haven't condensed by the time it hits the filter and gets through.

DR. ZWEIDINGER: We have initiated some basic studies in that area. The biggest problem we have is trying to collect gas phase materials without introducing artifacts.

DR. ALBERT: I would raise the issue as to what is meant by artifacts For example, the whole cigarette business has been investigated in terms of condensate. There may be artifacts, but wouldn't they be comparable artifacts in comparing these various materials?

DR. BRADOW: I have heard these discussions before. Let me point out the significant differences between the nature of cigarette smoke condensates and diesel condensates, or, in fact, gasoline condensates.

First of all, in the effluent of a gasoline engine, the exhaust nitrogen oxide content would normally be between, say, 200 and 1000 parts per million by volume. When we condense this material, we condense a great deal of combustion water. In the combustion water there will be accumulated a portion of the NO_2, and very likely an equilibrium amount of the HONO, the nitrous acid which exists whenever there is NO_2 and NO present through water vapor.

Experimentally, we find that a condensate of this type is capable of quantitatively extracting the olefinic hydrocarbons from the gas phase and what appears in the solution, then, in the water solution, in the combustion solution, is a mixture of compounds whose structures have been reasonably well identified. These materials include the addition products of nitrous and nitric acid for the olefinic hydrocarbons.

Now, when gasoline engine exhaust is allowed to expand into the ambient air, none of these--first of all, the olefinic hydrocarbons stay in the gas phase, the formation of the nitrous acid occurs, but the addition does not occur, so you do not find in gas phase materials short of actually allowing the photochemistry to happen, you do not find in direct emissions nitro olefin, nitro alcohol, and nitro nitrate esters in the gas phase. They are not present. They are present in the combustion condensates.

In the case of cigarette smoke condensate, possibly the issue may be more relevant. After all, one, as I do, breathes in the total combustion gases from a cigarette. These same acid gases are very likely extracted by the water, the high relative humidity condensing water in a person's lungs, and this simply doesn't happen with an automobile exhaust.

So the question of whether the materials which we see in this condensate is artifactual in the one case and not in the other may be very relevant. We are positive that olefinic hydrocarbons emitted from the tailpipes of passenger cars exist as olefinic hydrocarbons in the ambient air. They do not in the case of water condensates and coldtrap condensates.

The nitro compounds we're talking about here are toxic materials, and considering the number of nitro compounds which are also mutagenic materials, we are really sure that this is an artifact. In fact, this has been so well documented for such a long period of time that early, 25 years ago, when people started to work on automobile exhaust, we abandoned coldtrapping as a reasonable means for selecting samples--concentrating samples of automobile exhaust for purposes of characterization studies.

The same thing happens in my experience with diesel exhaust also. We do form in solution because of very rapid ionic reactions which occur in water solutions with acid. We find very similar artifacts with diesel, and these just do not exist with gas.

DR. ALBERT: Are you content that the stuff that gets through the filter is not going to be of biological importance?

DR. BRADOW: No, sir, but there's not very much we can do about it. In the case of the other collection procedures we have attempted to use, we've had also great difficulty.

For example, a classic way of determining trace components in ambient air is to use a low temperature porous polymer trapping medium, such as, for example, XAB$_2$, or protho 102, or 10XGC, in order to concentrate materials for chemical analysis out of a large volume of ambient air.

Our experience with automobile exhaust has been entirely negative for these substances. For example, in the case of diesel exhaust, experiments with protho 102, which seems to be the least reactive of these materials, have indicated that substances are formed by the reaction to nitrogen oxide with the porous polymer itself. Extraction of a porous polymer trap initially to exhaustion ends up with a very clean material upon which organic material can be collected.

When we do this with diesel engine exhaust, and subsequently extract diesel engine materials out of the forced polymer trap, we acquire a sample which has three times the mass which could be present on the sample provided the whole hydrocarbon content, the whole organic content, measured by FID in the automobile exhaust were trapped.

Further, the material which has been eluted from the trap is toxic, too toxic to test. In other words, we get twice as much artifacts by reaction of porous polymer as we got material trapped, including the inner hydrocarbons.

The material that we got this through we attempted to fractionate using the same fractionation procedure, hoping that it would be in the OXY cut or in some cut in which a mutagen was not present. This turned out not to be the case. The entire extra material ended up in the same fraction chemically that the active fractions in the diesel exhaust were. This same experience has been repeated with XAD2 and 10XGC, so the porous polymer trapping procedure seems to be completely out.

We have been working recently with activated charcoal. Activated carbon, lampblack extracts, are about as mutagenic and have about the same mass yield relative to the carbon, as does diesel exhaust. Every lampblack sample we found has been that way.

When we extract these lampblack samples and attempt to dry them out, we get a very surface area lampblack from which, once diesel exhaust is trapped on it, we have not been able to recover any of the materials. I'm not saying that it is not possible ultimately to do this, to find a qualified procedure to concentrate and test the gaseous material, but man, we're not going to do this in a short term. At this point, we are not there, close to there, and we are beginning to exhaust all funds.

DR. FLAMM: That was very useful, vitally important. Dr. Campbell?

DR. CAMPBELL: I am from the Health Effects Laboratory in Cincinnati that has been alluded to this morning. Our plans were to cover this particular item as one piece of our overall program which is primarily inhalation toxicology and oncogenicity, if you will, of diesel exhaust.

This is what you might call a comparative oncogenicity test of diesel soot comparing diesel soot samples along with the other environmental pollutant materials that have been alluded to as well, and which are being tested under the matrix system by Dr. Huisingh and Dr. Nesnow and others by intratracheal injection and so on.

I call it comparative oncogenicity because I'm not sure it can be called strictly comparative potency tests inasmuch as we're using the strain A mice in 55 of the group, and injecting only 1 dose level over a period of time of all these materials at approximate maximum tolerated dose when we find out what that is going to be.

It is the strain A injection model which involves multiple injections, I belive three times a week over something like 8 weeks, and then holding for another 16 or so weeks, and then the mice are sacrified at 9 months, counting then the tumors per animal in the lung, and animals with tumors, and performing pathology, body weight, and other pathology on the animals at that time.

This should provide us some direct evidence of oncogenicity, whether it is promotion, induction, whole carcinogenicity, or whatever, but the induction of tumors in these animals by injection.

It should complement, as I mentioned, the intratracheal studies, the inhalation studies in strain A and hamster, the skin tests, and the in vitro tests in whatever way possible, providing that data base.

That's about all I have on that. The rest of our material I plan to give a litle later on, and this will just save some time there.

DR. FLAMM: Does the panel have any questions of Dr. Campbell at this time? (No response.)

Well, that being the case, unless the general chairman has a problem with this, I think it might not be a bad idea if we were we to break for lunch at this point, and I would like to ask you to get back 15 minutes earlier than the schedule shows. That will be around 1:15.

(A luncheon recess was taken.)

DR. PEPELKO: I am William Pepelko from the Health Effects Research Laboratory in Cincinnati. Dr. Kirby Campbell and I are going to attempt to cover the health effects research that is being carried out in diesel exhaust in Cincinnati.

We are involved primarily with whole exhaust inhalation exposures, as opposed to the in vitro work that's done at Research Triangle Park.

I'd like to show you a few slides,* about our facility, mainly to point out the capabilities and limitations of this facility.

This facility is located about 4 miles from our main research building. It's in a fairly isolated location, and it's a good place to carry out this type of work. The high bay area is where our chambers are. On the left-hand side are offices and laboratories. Could I have the next slide* please?

This is a close-up of one our dynamometer base plates with an engine on it. This is a Nissan diesel engine, which we used in our previous short-term study and are also using in our present exposures.

We have, at present, two dynamometer base plates. Two engines are mounted on one of these base plates, which we can alternate back and forth, so we can service one engine while the other engine is running. The other base plate will be used by NIOSH for a mine diesel study.

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* Not available for reporduction in this report.

This is just an overhead view of our engines. The engine on the right is a gasoline V-8 engine, which has since been removed. Both our engines are Nissan diesels--next slide,* please.

This is just a slide to show a schematic of our system. I won't go into any details on that--next slide.*

I had a slide to show the chambers which will probably show up at the end of the carrousel. But what essentially we have are 24 exposure chambers. These chambers are either 5 feet square or 4-1/2 feet square with an interior volume of about a 100 cubic feet. At present we are constructing 12 additional chambers of similar size, which will be used on a 2-year NIOSH study to study diesel exhaust from mine diesels, with animals exposed to either diesel exhaust or a combination of diesel exhaust plus coal dust.

We are presently attempting to expand our facility and we would like to add an additional 18 chambers. We are limited, as was brought out in the talks this morning. In order to compare gasoline and diesel engines, it is desirable to study whole exhaust emissions. At present we don't have sufficient chambers or the dynamometer base plates to do a direct comparison between diesel and gasoline engines.

In the past there have been recommendations that we study more than one level of exposure. Again, we don't have the facilities to do this. All 24 of our chambers are presently in use with the studies that we're going to describe this afternoon.

I am going to discuss noncarcinogenicity studies while Kirby Campbell will cover the carcinogenicity studies which we are performing in Cincinnati. Our major emphasis is on cancer.

Kirby will speak first, then I'll discuss studies in behavior, pulmonary toxicology, and biochemistry. So if Kirby would like to come up--

DR. CAMPBELL: Bill got to have all the nice picturesque slides, and the ones I have are somewhat less interesting. If I may have the first one, please? (Slide #12.)

This is just an overview of what I intend to talk about: a little bit of the engineering and the engineering conditions that we've set up for conduct of these studies; the aerometry; the monitoring of the exposure atmospheres, with some of the conditions and results that we have so far; and the toxicologic investigations dealing with direct and indirect oncogenicity testing. The nononcogenicity toxicity will be covered primarily by Bill Pepelko.

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* Not available for reproduction in this report.

TADE - II DIESEL EMISSION TOXICOLOGY
PROJECT HERL/EPA/CINCINNATI

ENGINEERING: GENERATION, DISTRIBUTION OF TEST ATMOSPHERES

AEROMETRY: CHARACTERIZATION OF EXPERIMENTAL ATMOSPHERES

TOXICOLOGIC INVESTIGATIONS:

DIRECT AND INDIRECT ONCOGENICITY-RELEVANT TESTS

NON-ONCOGENICITY TOXICITY

COMPARISON OF DIESEL- AND GASOLINE-EXHAUST INHALATION TOXICITY DATA TO DATE.

12

At the end I'd like to talk a little bit about the comparative data that we have, or at least quasicomparative data that we have so far on the toxicity of diesel compared with previous gasoline exhaust inhalation studies. May I have the next slide, please? (Slide #13.)

We have two Nissan CN6 light-duty, 6-cylinder, 4 cycle, 3244 cc. displacement engines with a compression ratio of 20:1. And the difference between the present diesel engines and those that we did the short-term pilot study on is that the engines we have now have a low smoke head design. The air intake to the engine, as well as the air supplied for dilution of the exhaust and distribution to the chambers, is purified with CBR and particulate filters. The dynamometer is an Eaton-Dynamatic.

Coupled to Chrysler torque-flight transmissions and the pair of diesel engines is an air gap, eddy current, dual rotational dynamometer, with power absorption characteristics to simulate a load of the 4,000-pound moving vehicle. The engines are operated on the Federal short cycle, 125 seconds in length, with 9 components, including idle, acceleration, deceleration, and cruise. The fuel being used is a number 2 diesel. I believe Gulf is the brand that we're using, purchased from a local distributor.

The fuel consumption is running at about a quarter of a pound per mile, about 0.06 pounds of fuel per cubic meter of exhaust and about 0.003 pounds per cubic meter of exhaust in atmosphere in the exposure chambers. Another fuel consumption figure is 5 to 6 pounds per hour.

ENGINEERING

ENGINES:	NISSAN CN6-33, LIGHT DUTY, 6 CYL., 4 CY., 3244 CC DISPL. CR 22:1, MBH 92 @ 4000; LOW SMOKE HEAD DESIGN; PURIFIED, CONDITIONED AIR INTAKE.
DYNAMOMETER:	EATON-DYNAMATIC MOD. 758-DG, COUPLED TO CHRYSLER A-727 TORQUE-FLIGHT TRANSMISSION; AIR GAP, EDDY CURRENT, DUAL ROTATIONAL, WATER COOLED; POWER ABSORPTION 50 HP AT 1,600-10,000 RPM; 450 LB FLYWHEEL TO SIMULATE LOAD OF 4000 LB MOVING VEHICLE.

ENGINE OPERATING CYCLE: FEDERAL SHORT CYCLE, 125 SEC, 9 COMPONENTS, INCL. IDLE, ACCEL., DECEL., CRUISE.

FUEL: NO 2 DIESEL

FUEL CONUMPTION: 0.26 LB/MI, 0.08-0.06 LB/M^3 EXH., 5.1-6.3 LBS/HR.

EXHAUST DILUTION FACTOR: 12-28 AIR TO EXH. (APPROX. $\underline{X}$, 17)

ENGINE OUTPUT (EXH. FLOW): 48-55 CFM

CARBON BAL. RATIO (EMISS./FUEL): 0.82-1.06

CHAMBER TEST ATMOSPHERE TSP CONC (PROTOCOL): 6-7 MG/M^3.

13

The exhaust dilution factor has varied from 12 to about 22, clean air to raw, fresh exhaust; the approximate mean, as of about now, is about 17. One of the reasons for this variation is that we had a steadily increasing output of particulate material.

On the bottom line you see the design test atmosphere. The total suspended particulate concentration we have decided would be 6 to 7 milligrams per cubic meter. In order to maintain this level, with the increasing output of particulate from the engine, we've had to increase our dilution air ratio so that--this accounts for the factor increasing from 12 to about 22. We have an overall average dilution factor now of about 17.

The engine output exhaust, in terms of exhaust flow, is about 48 to 65 cubic feet per minute. And the carbon balance ratio computed to date has run about 0.8 to 1.06 (ratio of emission to fuel).

Okay, can I have the next slide, please? (Slide #14.)

AEROMETRY-CHAMBER ATMOSPHERE MONITORING

Automatically cycled: CO, CO_2, NO, NO_2, THC, SO_2

Other: CH_4, Non Meth HC, C_2H_6, C_2H_4, CHO's, NH_3, O_3, particulates (tot. mass, $SO_4^=$, NH_4^+, NO_3^-, Cl-, size), temp., rel. humidity.

Automatic data analysis control, acquisition, storage, statistical analysis for 6 gases by Hewlett-Packard 9830 electronic calculator with electrotronic controls and floppy disk memory. Selected period statistics available at will, weekly automatic, for CO, CO_2, NO, NO_2, THC, SO_2.

Analytic Principle/Instrumentation: NDIR/Beckman 865 (CO, CO_2; chemiluminescence/TECo 14T (NO, NO_2); FID/Beckman 400 (THC, amb.), Beckman 402 (THC, 350°F); Gas chromatography/Beckman 6800 (CH_4, NMHC, C_2H_6, C_2H_4); pulsed UV fluorescence/TECo (SO_2); chemiluminescence/Meloy 320 (ozone); phenol-hypochlorite/Beckman DB (NH_3); DNPH/GC/Perkin-Elmer 900 (CHO's); Pallfex filter gravimetry (TSP mass); ion chromatography/Dionex 14 (particulate ions: $SO_4^=$, NH_4^+, NO_3^-, Cl-; visual/SEM (partic. size, vis.); liq. chromatography (PNA's).

14

The components carbon monoxide, carbon dioxide, nitric oxide, nitrogen dioxide, total hydrocarbons and SO_2 are automatically monitored by a Hewlett-Packard 9830 calculator-operated data acquisition system.

Other components that are measured by other means, either by instrumentation or wet chemical methods, include methane, nonmethane hydrocarbons, ethane, ethylene, aldehydes, ammonia, ozone, and particulates, including total mass size and the ions (sulfate ammonia, nitrate, and chloride). We are also monitoring temperature, and relative humidity and controlling these within fairly tight limits within the exposure chamber.

The automatic data analysis control, acquisition, storage and statistical analysis for the gases named above are done by the Hewlett-Packard calculator system, which has a floppy disk memory. Data acquired can then be recalled and analyzed statistically at any time we need.

Analytic principles and instrumentation I have shown here, and if anybody wants to go into that, we can, perhaps at a later time. Could I have the next slide please? (Slide #15.) Let's get this thing moving on.

RANGE OR MEAN OF WEEKLY AVERAGE POLLUTANT CONCENTRATIONS
ACROSS CHAMBERS (DATA AVAILABLE AS OF 10 WEEKS)

POLLUTANT COMPONENT		TEST CHAMBERS (13-16)	CONTROL CHAMBERS (1-12)
CO_2	VOL%*	0.16 - 0.40	0.03 - 0.05
CO	PPM*	10.4 - 26.8	1.7 - 3.8
TCH	PPM*	7.0 - 10.8	2.4 - 4.6
NO	PPM*	8.7 - 15.3	0.05 - 0.40
NO_2	PPM*	2.4 - 5.0	0.03 - 0.09 (0.71?)
SO_2	PPM*	1.3 - 4.4	0.29 - 1.64
TSP	MG/M^{3+}	3.2 - 9.5 ($\bar{X}$ 6.7)	
PARTIC. DIA., VIS. µM**		0.05 (PRIM) -1.5 (AGGREG.)	

* N = 3-7/CH./DAY
+ N = 5/WK (ALL CHAMB.); PALLFLEX.
** NUCLEOPORE; SEM; AED YET TO BE DETERM.

15

The overall average, as of about 10 weeks of operation, inasmuch as the study was officially begun on the 18th of September, is reflected by these weekly mean--or range of weekly mean--data here for the major components, CO_2, CO, total hydrocarbons, NO_x's and so on.

They are reasonably comparable to those that we found in the pilot study. And, I think I recall that the particle output of the catalytic study that we ran before the pilot diesel study--it was of about the same order as that which we are getting now. But, of course, as we've mentioned, those would be primarily sulfuric acid, a completely different character from the total particulate that we have now. The corresponding levels of these components in the control chambers are shown here in the right column.

The particulate diameter information that we have so far is entirely visual, based on scanning electron microscopy, and that appears to be in the range of about 0.05 micrometers for the primary particle up to an aggregate size of about 1-1/2 micra, which is very close to what Ron Bradow mentioned this morning. We do not yet have an aerodynamic diameter for these particulates, but we're trying to get such a measurement underway.

DR. JONES: Do you have an idea of what fraction is aggregated of the mass? Do you have a feeling for that?

DR. CAMPBELL: I think I might have a transparency showing that. I didn't make a slide of it. But you'll see a lot of individual primary particles on this nucleopore filter base, several small, short chains or very small aggregates, then increasing-sized aggregates up to very few of the larger-sized range. So, it's quite apparent that at least the visual median diameter is well below a micron, and of course respirable. We have not yet attempted to take a figure for density and recalculate an aerodynamic mass diameter for these things as yet.

The particulate mass levels that we're measuring are measured on Pallflex filters of, I believe, 47 millimeter type size. Next slide, please. (Slide #16.)

DIRECT ONCOGENICITY TEST (1)

Experiment: Tumor induction, especially lung

Animal: Syr. hamsters, F

Group Size: N 100

Route exposure: Inhalation

Duration exposure: 20 months (near-lifespan)

Measurements: Tumors/anim. and animals with tumors; pathology, body weight, general observation (P/BW/GO).

Comment: Direct Evidence of oncongenicity; complements I.T. tests (Saffioti model) elsewhere.

16

Okay, now into the onco-relevant biology--the first experiment is a test of tumor induction in female Syrian hamsters. The group was about 100 for the control and 100 in the diesel test atmosphere. I should mention that we are using only one diesel atmosphere, which is the diluted, nonirradiated diesel. In the pilot study we had included irradiated exhaust as well, and in our previous gasoline studies we included irradiated. But to make the most of our chambers in this situation, we decided to drop the irradiated feature.

The exposure duration for this study is expected to be about 20 months, their life span, or at least, quote, life span for the hamster, with measurements of tumors per animal and animals with tumors, pathology, body weight, and general observations, of course, along with it, which is designated here as P/BW/GO. You'll see that notation on the other slides as well.

This should give some kind of direct evidence of oncogenicity. And, we feel that it complements the intratracheal tests on Syrian hamsters that are being conducted elsewhere that you will hear more about a little bit later. Could I have the next slide, please? (Slide #17.)

DIRECT ONCOGENICITY TEST (2)

EXPERIMENT: TUMOR INDUCTION/PROMOTION, ESPECIALLY LUNG

ANIMAL: STRAIN A MICE, M

GROUP SIZE: N = 400^+

ROUTE EXPOSURE: INHALATION

DURATION EXPOSURE: 8-9 MOS. (WEAN TO 9 MOS.)

MEASUREMENTS: TUMORS (EXPEC. LUNG) PER ANIMAL AND ANIMALS WITH TUMORS; P/BW/GO

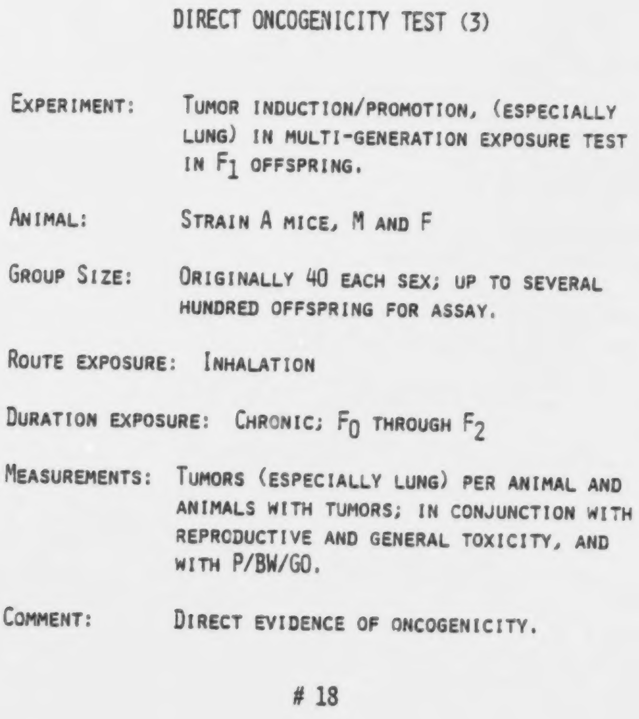
COMMENT: DIRECT EVIDENCE OF ONCOGENICITY; COMPLEMENTS COMPARATIVE I.P. TEST IN MICE.

17

The second direct inhalation oncogenicity test is one of tumor induction and/or promotion, especially of lung tumors, using the male strain A mouse.

We are using here a group size of 400-plus, starting out with more than 400, so as, hopefully, to maintain a design level of 400 per exposure and control group, with an overall duration of 8 to 9 months in the atmosphere. I should mention that obviously there would be no data--that is, probably no useful completed data--from the hamster study, since it is such a long-duration study, by the magic deadline of next fall. However, from this mouse study, we would expect to have some completed information by next fall. Again, we would expect to measure tumors per animal and animals with tumors and the other ordinary tag-along parameters.

Again, we feel that this study would provide rather direct evidence of an oncogenic influence of some sort, but whether it would be by promotion or initiation mechanisms, I don't think could be necessarily distinguished by the design. We feel that it complements, again, the comparative intraperitoneal test done in strain A mice (the same strain). Could I have the next slide, please? (Slide #18.)



DIRECT ONCOGENICITY TEST (3)

EXPERIMENT:	TUMOR INDUCTION/PROMOTION, (ESPECIALLY LUNG) IN MULTI-GENERATION EXPOSURE TEST IN F_1 OFFSPRING.
ANIMAL:	STRAIN A MICE, M AND F
GROUP SIZE:	ORIGINALLY 40 EACH SEX; UP TO SEVERAL HUNDRED OFFSPRING FOR ASSAY.
ROUTE EXPOSURE:	INHALATION
DURATION EXPOSURE:	CHRONIC; F_0 THROUGH F_2
MEASUREMENTS:	TUMORS (ESPECIALLY LUNG) PER ANIMAL AND ANIMALS WITH TUMORS; IN CONJUNCTION WITH REPRODUCTIVE AND GENERAL TOXICITY, AND WITH P/BW/GO.
COMMENT:	DIRECT EVIDENCE OF ONCOGENICITY.

18

The third direct oncogenicity test, again, is in strain A mice. But it's in strain A mice which are offspring of a multigeneration study conducted for a determination of potential effects on reproductive function. The F_1 and F_2 mice pups from this study should give us a fair number; since we're initially starting with 40 of each sex in the F_0 generation, we would expect to accumulate quite a number of pups. These pups will be exposed from the time of birth up to a considerable age before sacrifice. Again, tumors per lung and animals with tumors, in conjunction with reproductive and general toxicity in a multigeneration design. May I have the next slide, please? (Slide #19.)

COMPARATIVE ONCOGENICITY OF DIESEL SOOT AND OTHER ENVIRONMENTAL POLLUTANT MATERIALS

ANIMAL: STRAIN A MICE, M

GROUP SIZE: 55

ROUTE EXPOSURE: INTRAPERITONEAL INJECTION, APPROX. MAX. TOLERATED DOSE.

DURATION EXPOSURE: MULTIPLE INJECTIONS OVER SEVERAL WEEKS, HOLD TILL 9 MOS.

MEASUREMENTS: TUMORS (ESPECIALLY LUNG) PER ANIMAL AND ANIMALS WITH TUMORS; P/BW/GO.

COMMENT: DIRECT EVIDENCE OF ONCOGENICITY; COMPLEMENT I.T., INHAL., SKIN, AND *IN VITRO* TESTS.

19

Now, this is the one that I talked a little bit about this morning. There isn't much more to say about it. It's the intraperitoneal injection of strain A mice, using the same test material--that is, extracts of the coke oven emission, the roofing tar, and diesel emissions, and also the cigarette smoke condensate--as being supplied to the other testing groups. This is just another "complemental test," because it wasn't being done elsewhere by this particular route. We would be using, of course, a level which is shown to be in the neighborhood of the maximum tolerated dose, probably, which is yet to be determined for these materials. Okay, next slide, please. (Slide #20).

ALKYLATED HEMOGLOBIN AMINO ACIDS

ANIMAL: CATS ± OTHER

GROUP SIZE: AS APPROPRIATE UP TO 27

ROUTE EXPOSURE: INHALATION

DURATION EXPOSURE: UP TO 4 MONTHS

MEASUREMENTS: ALTERED (ALKYLATED) HEMOGLOBIN AMINO ACIDS (HPLC SEPARATED; DETECTED BY FLUORESCENCE).

COMMENT: IN VIVO INDICATOR OF MUTAGENIC/ONCOGENIC EXPOSURE.

20

Now, in addition to the more direct types of tests, we thought that we should look into some indicator endpoints in in vivo-exposed animals to fill in that gap between direct inhalation induction testing and those that are indicated by in vitro testing methods to see if, again, that we can provide some additional data that will hang together.

In the cats that are being exposed for primarily pulmonary function and biochemical purposes, we propose to apply a study of altered hemoglobin amino acids in the blood of these cats at various periods of exposure duration. This, I am told, is a rather new indicator of mutagenic-oncogenic exposure, somewhat of a dosimeter, in that if the hemoglobin amino acids are alkylated, they are alkylated by compounds likely to be mutagenic or carcinogenic.

So this would just fit into more of this whole scheme. I think there are some of the parameters of this study that just haven't been completely worked out yet. The method is being worked up now by an investigator recently joining our organization. But we thought it would be a timely thing to try to do in the cats used for other purposes. Another thing related to a question that was brought up this morning is also a possibility, but I don't have a slide on it. It would be to look at altered germ cells in animals exposed by inhalation to this diesel atmosphere. This would be a matter of taking aliquots of animals exposed for varying periods and looking at morphology of the sperm head, as I understand, which would be some indication of mutagenic potential. The next slide, please. (Slide #21.)

BIOTRANSFORMATION SYSTEM AND METABOLITES IN KEY TISSUES

Animal: Mice and/or rats

Group size: 20

Route exposure: Inhalation

Duration exposure: 3,6,9 months

Measurements: AHH, epoxide hydratase, cytochrome P-448, 450, metabolites

Comment: Indirect in vivo chemical indicator of muta-/oncogenicity potential.

21

This is a study in which we would look at some of the indicator bioactivation enzymes, cytochromes P-448 and 450, and some metabolites in tissues of, probably, primarily mice exposed for varying durations to the diesel exhaust. It may provide further indirect, but in vivo, chemical evidence of the potential for mutagenic or oncogenetic effect. Next slide, please. (Slide #22.)

CYTOGENETIC ALTERATIONS IN DIRECT-EXPOSED LUNG TISSUE

Animal: As appropriate (e.g., R, H, M)

Group size: As appropriate (e.g., 10-20)

Route exposure: Inhalation

Duration exposure: Subchronic and/or chronic

Measurement: Sister chromatid exchange rate in in vivo-exposed lung cells.

Comment: Indirect in vivo indicator of muta-/oncogenicity potential.

22

This refers to the area that Joellen mentioned this morning, where we have animals exposed by inhalation for subchronic or chronic durations, and examined by sister chromatid exchange or other methodology in lung cells urine, other biologic specimens from these animals for mutagenic/oncogenic potential. We have yet to work out the details of this area of work. But, we feel that it is worth doing, since it would at least afford an opportunity to try to relate in vitro test data that is being collected to the in vivo data that we hope to collect. Next slide, please. (Slide #23.)

IN VITRO MUTAGENICITY TEST OF DIESEL EXHAUST SOOT

Animal: Bacterial (Ames) and cell culture system.

Group size: As appropriate

Route exposure: *In vitro*

Duration exposure: Short term

Measurements: Revertants, SCE rates

Comment: *In vitro*, indirect indicator of muta-/oncogenicity potential

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We have collected soot samples from our chambers. Before the present study got underway, we collected some irradiated and nonirradiated diesel exhaust atmosphere soot from the exposure chambers (without animals in them) and have submitted them for in vitro testing by bacterial and cell culture methods. This was our last opportunity for an effort to distinguish between irradiated and nonirradiated diesel exhaust.

I don't think there is anything more that can or needs to be said about that, except perhaps that we also sent a sample for aqueous elution as well as organic extraction from the soot samples, then testing in a sister chromatic exchange assay in WI-38 and CHO systems. I do not have the report back from this, except very preliminary, sketchily and unofficially, but apparently there was some cytotoxicity in the system, but no good indication of an increased SCE rate. I do not want to try to relate anything to anything here yet. And, I don't think we should until we have more data and experience with these types of systems. Okay, can I have the next slide, please? (Slide #24.)

IMPAIRED RESISTANCE TO INFECTION BY MICROBIAL PATHOGENS (INFECTIVITY TEST)

ANIMAL: MICE, CR/CD-1, F

GROUP SIZE: 20 (REPLICATED AS APPROPRIATE)

ROUTE EXPOSURE: INHALATION (BOTH TEST AND INFECTIOUS AREOSOL ATMOSPHERES)

DURATION EXPOSURE: ACUTE (6H), SUBACUTE (2 WK), CHRONIC (3-12 MO).

MEASUREMENTS: LETHAL (± MORBID) RESPONSE TO INFECTIOUS EXPOSURE (STREPTOCOCCUS, ± VIRUS) FOLLOWING TEST (EXHAUST) EXPOSURE.

COMMENT: ALTHOUGH NOT DIRECTLY RELEVANT TO ONCOGENICITY, NEVERTHELESS TOXICOLOGICALLY IMPORTANT IMPLICATIONS FOR PUBLIC HEALTH.

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Now, the first of the nononcogenic toxicity work has been underway for a while, at least in terms of some acute and subacute experiments to test for an impairment effect on the resistance to bacterial infection. We have used the model that was developed originally by Dr. Ehrlich up at IITRI and Dr. Coffin at RTP, which is a very sensitive system that has shown enhanced susceptibility to infection at very low levels of, at least irritant, pollutants.

In the use of this method so far, we have had positive results with our diesel exhaust. Of course that should probably come as no surprise to anyone, because there are irritants in the diesel exhaust, including those that might be expected to play a role in causing this effect.

We have data that, to the extent one cares to, may be compared among experiments of the current study to those of the earlier pilot diesel study and then back to the still earlier catalytic gasoline exhaust studies. These tests are run in replication, and always with simultaneous controls, but have not been run simultaneously in all test atmospheres so as to permit an ideal, straight comparison of potency. If one compares pooled data thus far, there may be a suggestion of a diesel-gasoline difference, which I'll mention in a moment. If I could have the next slide, please. (Slide #25.)

COMPARATIVE TOXICITY TO DATE OF DIESEL AND GASOLINE (CATALYTIC) AUTOMOTIVE EXHAUSTS

A. CONDITIONS (DIESEL COMPARED TO CATALYTIC):
SOMEWHAT GREATER DILUTION RATIO, NO_2, ALDEHYDES, SO_2; LESS FUEL MASS PER CUBIC METER EXHAUST OR TEST ATMOSPHERE.

B. TOXICOLOGY:

1. PULMONARY HISTOPATHOLOGY: GREATER DEGREE OF LESIONS AND/OR PARTICULATE-LADEN MACROPHAGES IN DIESEL-EXPOSED CAT, RAT, GUINEA PIG LUNGS.
2. PULMONARY FUNCTION: GREATER DECREASE IN MEF_{10} OF DIESEL THAN CATALYTIC-EXPOSED CATS, AND WITH LESS TOTAL EXPOSURE. AIRWAY RESISTANCE INCREASED SIGNIFICANTLY IN GUINEA PIGS EXPOSED 4 WEEKS TO DIESEL, BUT NOT AT 6 OR 12 WEEKS IN THOSE EXPOSED TO CATALYTIC EXHAUST.
3. BEHAVIOR: SUPPRESSION OF SPONTANEOUS LOCOMOTOR ACTIVITY GREATER AND/OR MORE PROLONGED IN DIESEL THAN IN GASOLINE EXHAUST-EXPOSED RATS.
4. RESISTANCE TO INFECTION: APPARENTLY GREATER SUPPRESSION (EXCESS MORTALITY) IN MICE SUBACUTELY EXPOSED TO DIESEL THAN IN THOSE EXPOSED TO CATALYTIC EXHAUST.
5. SERUM ENZYMES: ALTHOUGH ELEVATED IN CATALYTIC EXHAUST-EXPOSED RATS, LDH AND GOT WERE REDUCED IN DIESEL EXHAUST-EXPOSED RATS.

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Okay. Now, we do have a few areas in which some conditions and effects of diesel exhaust exposure might be compared with those of catalytic gasoline exhaust. If we take as a basis the comparison of diesel back to catalytic exhaust, we see that in terms of the exposure conditions, there is a somewhat greater dilution ratio with the diesel test system, somewhat greater NO_2, aldehydes, and sulfur dioxide levels, and interestingly, a lower fuel mass per cubic meter of exhaust atmosphere tested.

The latter stems from calculations suggesting that the amount (mass) of diesel fuel used to produce a cubic meter of test atmosphere is about one-fifth to one-fourth that used in producing our catalytic gasoline exhaust. One might conjecture from this that if diesel fuel consumption ever got to the point of gasoline consumption on a mass basis, that equal toxicity of similarly diesel and gasoline exhausts in test atmospheres would suggest that a greater potential for inhalation toxicity would exist "out there" with the use of diesel than with gasoline power.

Under these conditions, in terms of pulmonary histopathology, we have seen a greater degree of irritant lesions and/or particulate-laden macrophages in the diesl-exposed cat, rat, and guinea pig lungs.

In terms of pulmonary function, we've seen a greater decrease in maximum respiratory flow in the diesel-exposed than in catalytic-exposed cats, and with less total exposure. I should mention that there were some differences in total duration of exposure and differences in the total duration of exposure per day in the diesel and gasoline studies.

In the diesel pilot study upon which most of this is based, the diesel exposure was for 20 hours per day, and in the catalytic exhaust study it was 16 hours per day. When I said "with less total exposure," I was referring to the fact that the exposure in the diesel exhaust atmosphere was for a shorter total duration in terms of weeks and total time than with the gasoline exhaust.

Also, airway resistance increased significantly in guinea pigs exposed to 4 weeks of diesel, but did not do so at 6 to 12 weeks in those exposed to catalytic exhaust.

In terms of behaviorial testing, there was a suppression of spontaneous locomotor activity that was greater and/or more prolonged in diesel-exposed than gasoline exhaust-exposed rats.

In terms of resistance to infection, there has been an apparently greater suppression--that is, excess mortality compared to controls--in mice subacutely exposed to diesel, than in those exposed similarly to catalytic exhaust.

And, in the biochemical, serum enzyme area, although we don't quite know what this means yet, there was an elevation in catalytic exhaust-exposed rats for LDH and GOT, but they were reduced in diesel exhaust-exposed rats.

Okay. That's all I have for the time being. If you'll permit us, I think Bill will continue with the rest of the nonoconogenic-related toxicity. We are trying to include a little broader toxicity base than only carcinogenicity-related tests in this whole program. And, now, you'll hear about the rest.

DR. FLAMM: Would any members of the panel care to--

DR. SELTSER: I just wanted to be sure I understood. These comparative studies were done at different times? These were not actual experiments done simultaneously?

DR. CAMPBELL: Yes, sir, they were. Although we have approached the capability to perform these studies concurrently, as many would like to have done now and should be done, of course, we have not actually performed them in that way. We were onto the gasoline, the fuel additives, the catalyst question, and so on. Our resources were devoted singly to those efforts, without making the comparison yet between diesel and gasoline at the same time and under the same exact conditions, with the same animal subjects, and so on. So, the studies have been sequential, but in all cases with internal controls, of course.

DR. SELTSER: The other thing I'd like to ask is, in a number of these slides, you showed a number of animals. Is this the total exposed, or does that include the control? Or how do you do that?

DR. CAMPBELL: That's number per exposure group, whether it be diesel or control, per cell. The number of 400 for the strain A mouse inhalation test means 400 controls and 400 exposed, which should give us a capability of detecting a 10 percent increase in incidence rate of tumors from a base of 35 percent. Now, if the base is lower than that, then our detectability increases; that is, we could detect a smaller increase from a lower base incidence.

In the hamster inhalation test of 100 per group, we have perhaps a bit higher increase in detectability. If the base incidence turns out to be zero to one percent animals with tumors, then we can expect to detect about an 8 percent increase in incidence.

DR. JONES: This question is somewhat philosophical in nature. The importance of the October 1979 decision point has been repeatedly emphasized. Thus, I am concerned about whether adequate data will be available at that point in time to permit a rational risk assessment. In particular, I am concerned that in many instances, including the whole animal exposure to raw diesel emissions, only one point on the dose-response curve was considered in the experimental design.

I wonder whether it might have been better to have more than one point on your dose-response curve, that is, exposure of either of the hamsters or the strain A mice or both to more than one dose and perhaps have waited until these results were avaialble before initiating the rather substantial number of indicator studies that are being conducted in parallel with the single dose point studies.

DR. CAMPBELL: You're right in many ways. We, also, would like to have been able to do a multidose design in all this. But to do so would cut down tremendously on the number of tests and other parallel work that we're doing.

So it comes down to a question of priorities. And perhaps this meeting can help identify some of these priorities. However, we have made some decisions, and we are underway. Some of our efforts may fail to provide risk-assessment-usable data by next fall, even with our shorter-term studies, but we felt some other longer-term toxicity should be checked, oncogenic and nononcogenic. Some adjustments in use of space for certain experiments might well be made, but to close the facility down for major engineering changes would set us back to where we would miss being able to produce data by the time of earliest need.

So, I can't really answer you very directly or provide any constructive, corrective approaches to it, except maybe to refer the question, either now or later, again, to Dr. Albert, who might like to say a few words on the one-dose level.

I know he said a good deal on multidose levels. But he has also said a few words on single-dose levels.

DR. ALBERT: Do you want me to say it now?

DR. CAMPBELL: Yes, if you would, please.

DR. ALBERT: Actually, I don't see why you couldn't use multiple doses, especially in the intraperitoneal route, in the strain A to get comparative potency data, rather than just restricting it to the maximum tolerable dose. That's a different situation than the availability of chambers for inhalation studies.

DR. CAMPBELL: It's availability of people there, I understand.

DR. ALBERT: I see. Well, all I can say is, it would be rather a simple thing to do, actually—to give several different doses by intraperitoneal injection. That's no big deal.

DR. CAMPBELL: It also increases the required sample size. And Ron Bradow is going to address that one pretty well.

DR. CORTESI: I don't understand, if you're going to linear-no threshold-extrapolate, what multiple doses do for you. I mean, why not just load all the animals into one point and draw the line through the heart?

DR. ALBERT: There is a variety of answers to that. Sometimes we do it, actually. But the approach that we have been taking is to use as low a dose or response level as the point of departure for the extrapolation.

I think, not uncommonly, one runs into the situation where you run so high on the dose that you essentially saturate the tumor response system. And you're sort of at a loss to begin, in terms of knowing whether or not you're justified in beginning the extrapolation at a very high level of response.

This comes up in the situation where you've got pretty close to 100 percent yield with, say, dose X. And you don't really know whether or not, if you were to give 1/10th that dose--whether you'd still get 100 percent yield. So starting an extrapolation from a very high level of response does provide some uncertainty. After all, the single hit dose response is really not linear, except at the lower end of it, because it tends to plateau off, as you begin to use up the responding subjects.

In terms of the response of the strain A by intraperitoneal injection, I think it would be just peachy if we got to that stage. I'm more inclined to be apprehensive about getting nothing. And it's very difficult to do comparative responses on the basis of no effects.

So, I'd take this single dose at a maximum tolerable level, if it produced some results that we could use to cross-compare. And, I might add that, at least my point of view was, that in terms of the intraperitoneal studies, it was more to cross-compare the agents with respect to their carcinogenic punch than to use the intraperitoneal route as a point of departure for extrapolation, because the route of exposure is so far from the realities of people getting exposed by the inhalation route, that I don't really think that even we could undertake to use it, but the chambers, I think have got the limitation of just a finite number of chambers. And, you can't run more, I take it, than the planned exposures.

DR. CORTESI: But, Dr. Jones' question--the probability of getting a response, you feel is low enough that you doubt that you would saturate the animal's system with the doses that are being given both in the chamber here and in the IP work, so that one dose really is a perfectly good experiment, in this case, for what you wish to do, should you use it for a risk assessment.

DR. ALBERT: Well, I think the chamber study was designed to produce something. And, given the limited numbers of chambers, the thought was to give as large an exposure as we could get away with, in the hopes that we'd get something.

Actually, I'd prefer to see the intraperitoneal done with a couple of different dose levels, in maybe a half and a quarter of the maximum tolerated dose, if the resources were there, just on the optimistic point of view that maybe you'd really get something substantial at the maximum tolerated dose.

DR. CAMPBELL: Thank you again. We'll see what we can do. I would like to see it, too. But, we do have to work with the people we have. And the one that I've approached so far on that one indicates that he would need more help, and we have a help-hiring problem.

DR. FLAMM: Dr. Goldman has a question.

DR. GOLDMAN: With regard to some of these enzyme assays, I wonder the extent to which the ingestion of the material in a long-term inhalation study might interfere with the results for the microgram of a body burden taken by inhalation with cats or rodents. And, how many are taken in by licking and ingestion? It is a problem, since both routes represent a source of selection which might interfere with the quantitation of this effect.

DR. CAMPBELL: I hope not, Dr. Goldman. And, I hope that somebody maybe can answer this. Perhaps we can devise a way to get around an answer to this, if somebody hasn't already solved it in the near future or even by now.

It would impact on interpretation, as you point out. It does not mean that we can do it a different way. So, we could find out the effect of this fur-licking exposure on the whole thing and maybe come up with a usable factor, some kind of an interpretive factor at least for the chronic inhalation study at this time.

And, I would just hope that somebody around might have a solution for that.

DR. ZEIGER: You've mentioned a number of endpoints, alkylated hemoglobin, microsomial enzyme induction, sister chromatid exchange and cytogenetics in vivo and also urine assays. Now, are these being done now, concurrently with the inhalation or intraperitoneal exposures? Or, are these planned? Or, are they sort of separate from the carcinogenesis studies?

DR. CAMPBELL: Most of those mentioned are not yet being done. They will be concurrent with the inhalation studies proceeding at that time. They are not underway yet. Some of them will involve tissues from animals that are under inhalation exposure, nondestructively.

Some of them will be separate animals exposed for a preset duration, with their own little niche in a chamber, all scheduled in with all the other animals that have to go in chambers and so on. So, not now, but they will be. And, they will have to be scheduled in with all the other occupants of those chambers.

DR. ZEIGER: Just one other point--will you be doing these same endpoints on the animals that are getting intraperitoneal exposure as well as those being under inhalation exposure?

DR. CAMPBELL: It has not been planned that way. The intraperitoneal exposure experiment was just an application of intraperitoneal strain A mouse model test, as Roy put it, to get a comparative punch assessment of the sample materials that are being provided to us.

DR. SELTSER: I'd like to know to what extent some of these data you're presenting in the noncarcinogenic studies, the two toxicity studies, are anticipated as being used in the risk assessment decisions that are to be made in December.

DR. CAMPBELL: There's a Bingo $64 one. Of course, we have some of our own ideas. I think this gets into kind of a philosophical area that might best be left for a different time, perhaps tomorrow or later today if we can get it in, because it is very far-reaching, and it has to do with agency policy.

And at that point I'm just going to cop out a little bit on agency policy. Maybe Dr. Cortesi can address that one.

DR. CORTESI: The question, first of all, is not relevant to this meeting. However, it is expected that the major worry that will occur in assessing these things will be the carcinogenic endpoint, from the point of view of what the general public health concern seems to be that we're focused on. Now, whether that is at the levels of exposure to these other effects that occurred and whether that is proper or not, as Kirby said, is a regulatory policy row and not a research data-gathering row. Thank you.

DR. CAMPBELL: You mentioned that the other ones were acute. They're not necessarily acute. We'll be detecting, perhaps, some short-term effects, naturally. But the whole idea of this thing is a rather long-term toxicity followup of a rather short-term pilot exposure, in which, indeed, we did find some indications of toxic effect.

This we proposed much earlier before they put us into the oncogenicity game. We did not do an oncogenicity emphasis study in the pilot study. Now we have this new area of emphasis. But we also feel that we should provide some toxicologic data, as well, of a different sort and over a long period, of some of the indicators that we have.

For example, I think Bill Pepelko can mention the pulmonary function. The changes we noted indicated a possibility of the development of long-term effects on the lung. So it isn't just an acute thing. We are doing long-term exposures to get at chronic toxicity problems.

I should mention that even though we did not attempt a definitive carcinogenicity study in the pilot project, we did put some strain A mice in, and we did get some equivocal data from that. We also injected intraperitoneally some strain A mice with some particulate material in aqueous medium. Those data were essentially completely equivocal.

DR. ALBERT: It wasn't extracted, though. It was a saline.

DR. CAMPBELL: It was a saline suspension. That's right, yes.

DR. FLAMM: Are there are any further questions from the panel?

(No response.)

DR. FLAMM: I think one of the things that we're really going to have to find out, with regard to this kind of work, is who and what kinds of studies are ongoing or planned that will relate considerations of exposure to considerations of dose.

If, in fact, all this research is to result in not just an estimate of the upper limit of risk, where one takes for granted that the risk is going to be fairly low, in the 10^{-5} area, for instance, where factors of 2 and factors of 3 mean very, very little--if in this instance it's higher than that, as it conceivably could be--although I have no idea what it might be--then refining our understanding of the relationship between human exposure, which is based on breathing ambient air with a certain level or density of particles and so forth and so on, to what humans are really exposed to, which is their true quarantinability and to do some of the dosimetry in mass, so that these studies can be related one to another.

I think it's really going to be awfully difficult to know what to do with the data unless it comes out as Roy was hoping it might, where everything sort of says the same thing. And then you clearly have no problem. And on that I would agree. But I'd be surprised if that were to happen. I think that this is something we probably will need to discuss tomorrow. I think other things are going to have to be built in, which provide better for an understanding of dose and provide for tying some of this data together so one can make heads or tails of it.

Having said that, I think it might be appropriate if we were to adjourn for 10 minutes and then return. And our next presentation, I understand, will take only about 10 or 15 minutes.

(Whereupon, a short recess was taken.)

DR. FLAMM: Ladies and gentlemen, can we come to order, please? The next speaker will be Dr. William Pepelko.

DR. PEPELKO: I will finish up our Cincinnati inhalation studies covering nononcogenic experiments. I'd just like to make a couple of comments why we feel this type of research is important, even though the overall emphasis is upon cancer. First of all, there is a chance that diesel exhaust does not add significantly to the risk of carcinogenesis.

This would bring us back to worrying about regulated and nonregulated gaseous pollutants. There is some evidence in the literature that the diesel particulate absorbs some of these pollutants, resulting in more efficient penetration along greater residence time, which can affect their toxicology, both in a quantitative and qualitative manner.

Since we're running behind schedule, I won't go into this in any more detail. I'll just describe our remaining experiments. (Slide #26.)

Our next experiment is a teratology study. In this study we're using albino rats and rabbits, 20 animals per group. With a normal rate of reproduction, this should result in at least 100 fetuses per group.

TERATOLOGY

HYPOTHESIS: DIESEL EXHAUST EXPOSURE RESULTS IN ABNORMAL DEVELOPMENT

ANIMALS USED: ALBINO RATS AND RABBITS

NUMBERS PER GROUP:

RATS - 20 CONTROLS - 20 EXPOSED
RABBITS - 20 CONTROLS - 20 EXPOSED
THESE NUMBERS SHOULD RESULT IN AT LEAST 100 FETUSES/GROUP

MEASUREMENTS: FETUSES ARE EXAMINED FOR ABNORMALITIES

DESIGN: PREGNANT RABBITS AND RATS WILL BE EXPOSED TO DIESEL EXHAUST DURING THE CRITICAL PERIOD FOR DEVELOPMENT OF ABNORMALITIES. THEY ARE SACRIFICED JUST PRIOR TO TERM FOR EXAMINATION OF FETUSES.

REPORT: JUNE 1979

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These animals will be exposed during the critical period of gestation for the development of abnormalities. This is a fairly straightforward, standard teratogenesis study.

Okay, we can go to the next slide, please. (Slide #27.)

Our next study will be a reproduction study. In this study we will use strain A mice. As Kirby Campbell has mentioned earlier, some of the offspring of these mice having been exposed to the diesel exhaust from conception will be used in a carcinogenesis study. In this study we are going to have a total of 40 males and 40 females initially. This will be a three-generation study, F_0, F_1, and F_2.

We're going to place the animals in the chamber at weaning age. They will be bred at maturity. Their offspring will either remain in the exhaust chambers or in the clean air chambers.

Measurements will include the number of offspring per litter, number of live births, stillbirths, litter weights, growth rates, survival rates, gross abnormalities, age at sexual maturity, length of estrus, length of gestation, maternal acceptance of offspring, mating behavior, and histological examination of testes and ovaries for abnormal sperm, abnormal ova, and so forth.

Forty F_1 females raised from weaning age in clean air and 40 raised in exhaust are bred at maturity to produce F_2 generation offspring.

REPRODUCTION

HYPOTHESIS: EXPOSURE TO DIESEL EXHAUST RESULTS IN ALTERED REPRODUCTIVE CAPACITY

ANIMALS USED: STRAIN "A" MICE

NUMBERS/GROUP: 40 MALES AND 40 FEMALES

MEASUREMENTS: NUMBER OF OFFSPRING PER LITTER, NUMBER OF LIVE BIRTHS, STILL BIRTHS, LITTER WEIGHTS, GROWTH RATES OF OFFSPRING, SURVIVAL RATES, GROSS ABNORMALITIES, AGE OF SEXUAL MATURITY, LENGTH OF ESTRUS, LENGTH OF GESTATION, MATERNAL ACCPETANCE OF OFFSPRING, MATING BEHAVIOR, HISTOLOGICAL EXAMINATION OF TESTES AND OVARIES

DESIGN: 40 FEMALES RAISED FROM WEANING AGE IN CLEAN AIR AND 40 RAISED IN EXHAUST CHAMBERS ARE BRED AT MATURITY.
F_1 GENERATION OFFSPRING ARE RAISED TO MATURITY AND BRED AS IN F_0.
F_2 GENERATION OFFSPRING RAISED TO MATURITY.
EXCESS OFFSPRING USED IN CARCINOGENESIS ASSAY.

STARTING DATE: 1979

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The animals not used for reproduction measurements will be maintained in the chambers until a minimum of 6 months of age, then examined for pulmonary tumors. The design is a little more complicated than this, but if there are any questions, we can discuss it later. Next slide, please. (Slide #28.)

EARLY DETECTION OF LUNG DAMAGE

HYPOTHESIS: SHORT-TERM EXPOSURE DAMAGES LUNG INTEGRITY

ANIMALS USED: RATS

NUMBERS/GROUP: 20/GROUP

MEASUREMENT: I^{131} ALBUMIN IN LUNG LAVAGE

DESIGN: GROUPS OF RATS ARE EXPOSED FOR 1-5 DAYS TO DIESEL EXHAUST FOLLOWED BY SACRIFICE AND ASSAY

REPORT: JUNE 1979

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The next study is a biochemical measurement: I^{131} albumin in the lung lavage. This is an extremely sensitive method of detecting changes in cell integrity. This method can predict the development of edema, for example. There were some indications from some of our other biochemical measurements of that cell repair and increased collagen formation was occurring. So we wanted to see if there was any indication of breakdown of lung integrity during early exposure that is repaired later on. Next slide, please. (Slide #29.)

CHRONIC LUNG DISEASE (BIOCHEMICAL PARAMETERS)

HYPOTHESIS: EXPOSURE TO DIESEL EXHAUST EMISSIONS RESULTS IN PULMONARY EMPHYSEMA AND/OR FIBROSIS

ANIMALS USED: STRAIN "A" MICE

NUMBERS/GROUP: 20

MEASUREMENT: COLLAGEN, COLLAGENASE, ELASTASE IN LUNG TISSUE

DESIGN: GROUPS OF MICE ARE EXPOSED FOR UP TO 1 YEAR TO DIESEL EXHAUST FOLLOWED BY SACRIFICE AND ASSAY

REPORT: JUNE 1980

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This is also a biochemical study, designed to detect chronic pulmonary emphysema and/or fibrosis, using strain A mice. Our measurements in this case will be collagen levels in lungs, collagenase, and elastase in lung tissues.

Twenty animals per group will be exposed for up to 1 year to diesel exhaust, followed by sacrifice and assay. Animals will be exposed 1, 3, 6, and 9 months. Next slide, please. (Slide #30.)

Our behavioral experiments will include quite a variety of measurements. We're interested in behavior since this is one area where we found quite dramatic changes during some of our initial exposures.

Both adult and neonate rats will be used for these exposures. Adults will be studied for voluntary activity.

Voluntary activity is one of the areas where we did find dramatic changes in response to diesel exposure. To measure voluntary activity, we placed the animals individually in activity wheels. The animals live in these activity wheels during the entire length of exposure, which in this case will be 16 weeks. We found in some of our previous studies that voluntary activity decreases to practically zero during exposure to diesel exhaust.

BEHAVIOR

Hypothesis: Exposure to inhaled diesel exhaust results in altered behavior and development

Animals Used: Adult and neonate rats

Numbers/Group: 13-18 per group

Measurement:

- Adults - Voluntary activity, forced activity, shuttle box learning, aggression, open field activity
- Neonates - Developmental data, spontaneous activity, learning forced activity, open field activity

Design: Adults exposed 16 weeks for measurements of voluntary activity using activity wheels, forced activity and shuttle box learning will be tested periodically using separate groups of rats exposed up to 6 months

Pregnate rats will be exposed to produce offspring for neonate testing

Reports: Interim June 1979

Final January 1981

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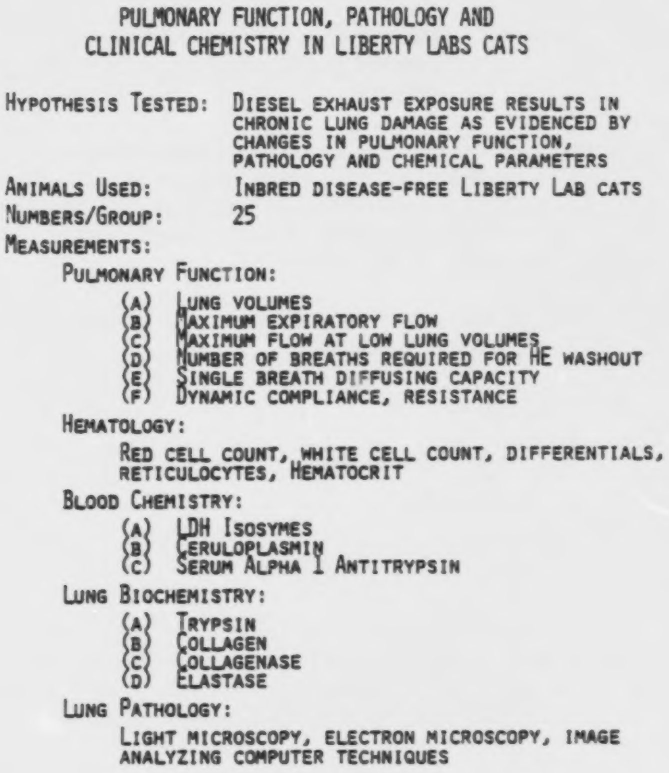
To measure forced activity, the animals were trained to run on a treadmill. They are tested monthly to the exhaustion point. Shuttle box learning is a type of avoidance test. A light comes on and the rat must run through an opening to the other side of the cage to avoid a shock.

Aggression will be tested by placing an exposed and a nonexposed animal on an electric grid and giving them a light shock. Presumably, the more aggressive animal will attack the less aggressive animal.

Open field activity is measured by placing the rats in a box on a grid on a square and measuring how many squares they cover in a particular time period.

With the neonates we measure spontaneous activity; developmental data, learning, forced activity, and open field activity again. We found in our previous studies that the rate of development may have been delayed in the neonates.

In the design of this experiment, the adults are exposed for 16 weeks for measures of voluntary activity and forced activity. Shuttle box learning will be tested for a period in several groups of rats exposed up to 6 months. Pregnant rats will be exposed to produce offspring for the neonate testing. Next slide please. (Slide #31.)



PULMONARY FUNCTION, PATHOLOGY AND
CLINICAL CHEMISTRY IN LIBERTY LABS CATS

Hypothesis Tested: Diesel exhaust exposure results in chronic lung damage as evidenced by changes in pulmonary function, pathology and chemical parameters

Animals Used: Inbred disease-free Liberty Lab cats

Numbers/Group: 25

Measurements:

Pulmonary Function:

(a) Lung volumes
(b) Maximum expiratory flow
(c) Maximum flow at low lung volumes
(d) Number of breaths required for He washout
(e) Single breath diffusing capacity
(f) Dynamic compliance, resistance

Hematology:

Red cell count, white cell count, differentials, reticulocytes, Hematocrit

Blood Chemistry:

(a) LDH Isosymes
(b) Ceruloplasmin
(c) Serum Alpha I Antitrypsin

Lung Biochemistry:

(a) Trypsin
(b) Collagen
(c) Collagenase
(d) Elastase

Lung Pathology:

Light microscopy, electron microscopy, image analyzing computer techniques

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The next area is primarily pulmonary function testing. In these studies we're exposing Liberty Lab cats, an inbred strain of disease-free very uniform type of animal.

Our hypothesis is that diesel exhaust exposure results in chronic lung damage, as evidenced by the various pulmonary function measurements that we make. We're exposing approximately 25 animals per group.

Some of the measurements being conducted on these cats include lung volumes, such as residual volume, functional residual capacity, total lung capacity. Some of these lung volume measurements, such as residual volume and functional residual capacity, will almost always increase with the development of emphysema.

We measure maximum expiratory flow rates to detect constriction of the large airway. Maximum flow rate at low lung volumes will give us an indication of constriction or blockage in the small airways, which usually occurs with chronic bronchitis and these types of diseases.

Number of breaths required for helium washout, again, gives us some indication of chronic lung disease with constriction or obstruction in the small airways.

Single breath diffusing capacity gives us an indication of thickening of the alveolar membrane, or decreased area of the alveolar membranes with emphysema. Dynamic compliance and resistance also estimate changes in stiffness of the lung and small airway constriction.

In addition to pulmonary function, we will carry out a variety of other measurements on these animals, including hematology, some blood chemistry and LDH isosymes, which is an enzyme used to detect cellular damage. By use of the isosymes, hopefully, we will be able to detect what organ is damaged.

There has been a question concerning the fact that these animals may get additional exposure on their food by licking themselves, which would tend to place blood chemistry, for example, in question, because we don't know the exact level of exposure. This may be a question we have to address later on in some of our discussions.

Following completion of the exposure--which is planned to last at least 2 years unless our program somehow changes--pulmonary function will be tested. Following pulmonary function testing, we would remove the lungs. Portions of the lungs will be used for trypsin, collagen, collagenase, and elastase measurements. We would do lung pathology on these animals, light microscopy, electron microscopy, and hopefully, some image-analyzing computer techniques. So we hope to get quite a wide variety of measurements on these animals. Next slide, please. (Slide #32.)

This is just a little bit on the experimental design. They'll be exposed for 2 years. That's all I have for now.

PULMONARY FUNCTION, PATHOLOGY AND CLINICAL CHEMISTRY IN LIBERTY LAB CATS

EXPOSURES: ONE GROUP WILL BE EXPOSED TO CLEAN AIR AND ONE GROUP TO EXHAUST FOR 2 YEARS. PULMONARY FUNCTION WILL BE TESTED PRIOR TO AND AFTER COMPLETION OF EXPOSURE, AT LEAST ONCE DURING EXPOSURE AND AFTER COMPLETION OF EXPOSURE.

PATHOLOGY AND LUNG BIOCHEMISTRY WILL BE CARRIED OUT AFTER COMPLETION OF 2 YEARS' EXPOSURE.

START OF EXPOSURE: 10/78

COMPLETION: 10/80

32

DR. SELTSER: Is there any information on gasoline emissions available for the kinds of things you were just describing?

DR. PEPELKO: Yes, we do have some information on this. And, in fact, at the end of our last session, I believe Kirby Campbell had made some comparisons between gasoline engine exhaust and diesel engine exhaust.

DR. SELTSER: None of these studies are planned for comparing diesel emissions with gasoline emissions?

DR. PEPELKO: Not directly.

DR. CAMPBELL: That raises the specter again. But, at the present time, we are not able to do a concurrent comparative study and provide needed data on time. Now, we do propose to add facilities if it gets blessed and all that sort of thing, so that this capability would be there again. But, at the present time, we don't have them.

DR. PEPELKO: As I say, presently we have 24 chambers. We are building 12, which NIOSH will use for the next 2 years. We are proposing to build 18 more. And, if we could get up to the level of 54 chambers, then we are getting to the point where we can really do some adequate comparison between gasoline and diesel and possibly also multiple levels of exposure.

DR. SELTSER: Twenty-four now being used to handle the studies you have just described?

DR. PEPELKO: Right, the studies Kirby Campbell and I have described. These experiments, for the most part, are ongoing. Cat exposures were begun as of last September.

DR. WILEY: Am I to understand that the chambers are the only limiting factor? Is that the limiting factor in making the comparative studies between the gasoline engine and the diesel?

DR. PELPELKO: That's the primary limiting factor. Of course, we have a limiting factor with manpower and equipment and so forth. We could put in a new dynamometer base plate and put a gasoline engine on it and dedicate some of these chambers towards exposure to gasoline exhaust, in order to make a direct comparison.

But then I'd like to emphasize, even if the decision was made to do this today, we couldn't have this change-over in effect for probably, I would guess, less than 6 months, which definitely would not give us the data that people need by the end of 1979. So there really just isn't the time to do this.

DR. CORTESI: The plumbing isn't available. I mean, the plumbing does not exist now to run two engines and put the exhaust from one engine into half of the boxes and exhaust from the other engine into the boxes. You'd have to build a new expansion--whatever the hell that thing is called--and run the pipes around it. So it's a major replumbing of the system as it stands now.

DR. PEPELKO: And these things take considerable time.

DR. LEWIS: I'd like to make one comment on the concentration of the materials in the experimental atmosphere, namely nitrogen dioxide, which is, as I belive Kirby said, was around 5 parts per million. Obviously, you know you're going to get dramatic results with that. You don't have to use a radioactive I^{31} albumin to detect the edema that will result.

The same thing would be true relating to the particulate at 6 milligrams per cubic meter. The TLV and the Federal standard for carbon black is 3-1/2 milligrams per cubic meter. And that's for a working man in an 8-hour day.

And, you know, you're really socking it to these animals. And to extrapolate from that down to what man gets in his ambient environment, I think you need both data points minimally.

DR. PEPELKO: Well, we are working near what we consider near the maximum tolerated doses. This wasn't entirely our decision. Perhaps, Roy Albert might have a comment on this.

DR. ALBERT: It was in order to maximize the chances of getting an oncogenic response. That level wasn't chosen in reference to the evaluation of these other changes.

DR. CAMPBELL: It just happens that the NO_2 is running a little higher than our previous pilot study. And in relation to our gasoline exhaust studies, it appears to be a characteristic--

DR. LEWIS: In our gasoline exhaust studies, my recall is that the atmospheric NO_2 concentration only ran about a half a PPM or less in the nonirradiated auto exhaust exposures.

DR. CAMPBELL: They were about 2 and 3, Trent.

DR. LEWIS: But prior to use of the catalytic converter, what were the NO_2 concentrations?

DR. CAMPBELL: Well, there was one back there about 5.

DR. FLAMM: Don Gardner is going to introduce the next speaker.

DR. GARDNER: I've been asked to tell you who I am. I'm Don Gardner, Health Effects Research Laboratory, Biomedical Research Branch, Research Triangle Park, North Carolina.

The presentations of this section will continue to look at the lung as the primary target organ for measuring the effects of the diesel exhausts.

We have chosen to use two different models for this. Both of them are noninhalation models and they are the Saffiotti and the so-called modified Saffiotti model.

Both of these models will permit us to give some answers to the questions that came up earlier and be able to provide some preliminary dose-response data. We'll be able to compare brands x, y and z, whatever they may be, for carcinogenesis and, using the Saffiotti method, be able to perhaps give you some data on the carcinogenicity within the time frame initially set, in 1979. This work will be done under contract with IIT Research Institute in Chicago. They've been doing this type of work for many years; however, this program just started in October. They will present the design of the proposed study and what they have done so far.

The first speaker will be Alan Shefner, Associate Director of Life Science Research Division, who will give the overview.

Alan.

MR. SHEFFNER: I plan on presenting a short overview of the program we will be conducting and I'll turn it over to Dr. Clinton Grubbs who is the principal investigator on this grant and he will go into a series of experimental details concerning technology and experiments we have started and those we plan on initiating shortly.

Our program was initiated at the beginning of October 1978 and includes a number of facets. One is the preparation of dosage forms of diesel exhaust particles and its dichloromethane extractable components in characterized suspensions and solutions.

In addition, for the Saffiotti model, we will prepare characterized ferric oxide preparations which will be coprecipitated or otherwise bound to diesel particulates, diesel exhaust extracts and other selected substances of epidemiological concern.

We're planning to use two animal models in our studies. The majority of our effort will be directed towards studies utilizing the Saffiotti model for intratracheal administration.

In this method, a suspension of the particles of interest is instilled directly into the trachea and lungs of the hamster. This method has been widely used since Saffiotti introduced it and various carrier particles and carcinogens have been tested in this manner.

We have used this procedure at IITRI since 1969 under programs sponsored by the National Cancer Institute.

The second model we are using in our program is as described by Schreiber and Nettesheim. It also uses the hamster as experimental animal.

In this procedure an ingenious device is used to apply the test material to a defined and reproducible area of the trachea. We have used this system since 1975, and together with Dr. Schreiber, have somewhat modified and refined the instillation apparatus.

The first slide shows some characteristics of both systems. (Slide #33.)

LUNG CARCINOGENESIS MODELS

(INTRATRACHEAL INSTILLATIONS)

SAFFIOTTI TECHNIQUE

1. TRACHEA AND LUNG
2. DATA AVAILABLE FOR A VARIETY OF CHEMICALS
3. DOSE-RESPONSE
4. KNOWN QUANTITY APPLIED
5. TUMORS IN OTHER SITES

LOCALIZED TECHNIQUE (SCHREIBER AND NETTESHEIM)

1. TRACHEA
2. LOCALIZED DEPOSITION
3. DOSE-RESPONSE
4. RAPID EVALUATION
5. LOW MORTALITY

33

The Saffioti technique uses application directly to trachea and lung. There is a background of data available for a variety of chemicals which have been tested which shows dose response relationships. A known quantity can be applied and systemic responses are found in that tumors also develop in other sites.

The localized technique has application directly to the trachea. It is a localized application over a very narrow area of the trachea. It also shows dose-response relationships to those compounds to which it responds. It has an advantage in that one can get rapid evaluation. Within 25 weeks one can define tumor incidence with particular carcinogens.

The low mortality refers to the fact that it is a relatively nontraumatic procedure and controls show good survival.

We propose to take advantage of the utility of each procedure. The long-term carcinogenesis trails will be carried out using the Saffiotti procedure. Both diesel particles and dichloromethane extracts will be tested in this system.

The Schreiber technique is proposed for shorter-term trial of the extract itself and in a cocarcinogenesis trial in combination with methylnitrosourea, a known carcinogen which is active in this model.

As Dr. Grubbs will show, this procedure, using localized administration, leads to development of tracheal lesions in a relatively short period of time and we intend to use it as an indicator system in which we will carry out only limited histopathology.

The timetable for carrying out both of these studies is shown in the next slide. (Slide #34.)

MILESTONES

	Localized	Saffiotti
10/78	Start dose range	
12/78		Start Dose range
1/79	Complete dose range	
2/79	Start short-term assay	
6/79		Complete dose range
8/79	Complete short-term assay	Start assay
10/79	Draft final report (short-term)	
12/79	Start long-term assay*	
11/81		Draft final report
2/82	Draft final report (long-term)	

* No funding.

34

All materials for the initial studies have already been supplied by Drs. Bradow, Zweidinger and Tejada of EPA.

We have initiated dose-range studies in both the localized and Saffiotti models with the dichloromethane extracts. We plan on starting the short-term assay by the localized method in February. We should have animals off by August; histopathology performed and a draft final report by October.

If the short-term assay by the Schreiber localized technique produces interesting results, one could consider a lifetime carcinogenicity study by this technology, but no funding has been requested for this as yet.

The Saffiotti study that is planned includes dose-range studies on the dichloromethane extracts which have already started and on the particle preparations which are in preparation now.

The dose-range studies should be completed by June of 1979 at which point we would review the data, try to pick a dose which would allow for good lifetime survival; start the long-term assay in August of 1979 and treat for 24 months.

May I have the next slide, please? (Slide #35.)

SAFFIOTTI INTRATRACHEAL INSTILLATION

TREATMENT	NO. DOSES
1. D.P.[A]	3
2. D.P. + Fe_2O_3	3
3. D.E.[B] + Fe_2O_3	3
4. B(A)P + Fe_2O_3	1
5. SAMPLE + Fe_2O_3	3
6. TREAT. CONTROL (Fe_2O_3)	1
7. UNTREAT. CONTROL	1

[A] DIESEL PARTICULATE

[B] DIESEL EXTRACT

35

The design of the experiments as they have come forward is as follows: The Saffiotti intratracheal instillation long-term carcinogenesis program provides for evaluation of diesel particulates suspended in gel saline; particulates bound to hematite; diesel extracts bound to hematite; and selected extract or condensate also bound to hematite.

Decisions have not yet been made as to whether it should be coke oven emissions or possibly all three of the materials of epidemiological concern, also bound to hematite and, of course, appropriate controls are also included.

That includes BaP-hematite as a positive control, a treatment control and untreated control groups.

The next slide shows the design of the modified intratracheal technique studies. (Slide #36.)

LOCALIZED INTRATRACHEAL INSTILLATION

Treatment	No. Doses
1. D.E.[A]	3
2. MNU[B]	1
3. MNU + D.E.	3
4. Solvent control	1
5. Untreated control	1

[A] Diesel Extract

[B] Methylnitrosourea

36

There is a three-dose level and assay of the diesel extract. An additional study in which the diesel extract and methylnitrosourea are given in a cocarcinogenesis mode, MNU control, solvent control and untreated control.

This model would be highly appropriate for studying dichloromethane extracts of the three materials which have been talked of as epidemiological controls.

For this model one must have reasonably soluble or extremely fine suspensions to utilize. It is not a model for the study of particulates.

I think that really covers the generalities of the program. I'd like Dr. Grubbs to talk about specifics.

DR. GRUBBS: Thank you, Alan.

I'd like to partially reiterate Alan's introduction so that we have a clear understanding of exactly what these two procedures are.

The two lung tumor models that I'll be talking about are also known as topical tumor induction methods. In a topical model, the test substance, or known carcinogen, is placed in direct contact with the target tissue, the main effect being produced at the initial point of contact.

The topical application models can be further divided into two models, that of localized tumor induction and that which is called the generalized respiratory tract exposure. In the former, or the localized tumor induction model, most or all of the carcinogen exposure remains confined to a preselected area or segment of the respiratory tract.

In the generalized respiratory tract exposure, all or most of the respiratory tract segments are exposed simultaneously at the time of instillation.

Again, as Alan has said, we are planning to use both models to study diesel fuel exhaust emissions for either carcinogenic and/or cocarcinogenic action.

The localized technique is also being called the tracheal washing technique. The generalized respiratory tract technique is the well-known Saffiotti method.

During the next few minutes, I would like to describe in detail the actual mechanics and equipment necessary for administering the compounds, the type of results that we can expect when known chemical carcinogens are used in the systems and finally to describe the preliminary studies that we have initiated at IITRI, using diesel fuel extracts during the last 2 months.

A few comments on the Saffiotti technique. The methodology consists essentially of the intratracheal instillation of a saline suspension of a particulated material to which fine particles of the carcinogenic chemical has been attached.

The mixed dust penetrates into the wall of the respiratory bronchials and alveoli, where they are engulfed by microphages and retained, hopefully, for long periods of time. This long period of time is necessary for the carcinogen to diffuse out, reach the epithelial cells lining the respiratory tract and bring about a neoplastic transformation.

Now, there's been numerous variations of the Saffiotti technique with regard to various chemical carcinogens, types of carriers, ratio of carrier to carcinogen concentrations, size of particles instilled, and also number and frequency of instillations.

However, I'd like to limit my discussion to perhaps the best known method, that which was originally described by Saffiotti using the carcinogen, benzo-a-pyrene, and the carrier dust, ferric oxide.

Saffiotti initially described two methods for preparing the BP-ferric oxide mixture for administration to animals, one in which the BP crystals of ferric oxide were ground together in a mortar until the crystal size of the carcinogen was equal to that of the ferric oxide.

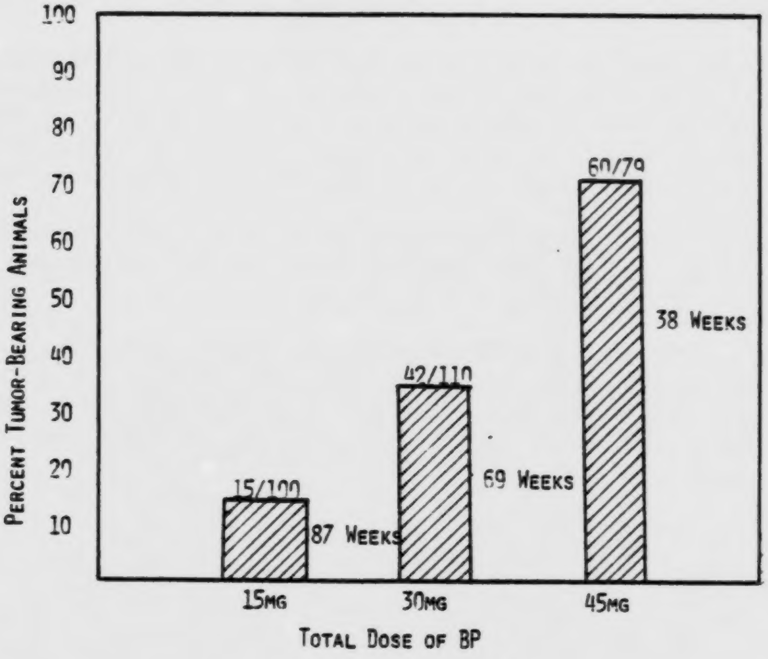
The second method called for preparing a concentrated solution of BP and acetone and slowly pouring the solution into distilled water containing the carrier dust. The carcinogen, during this procedure, crystallizes into very fine particles attached to the particles of the carrier dust.

At the end of this talk we'll show some photomicrographs of how these preparations look.

At the time of administration to the animals, the BP-ferric oxide is mixed with a 0.5 percent gelatin-saline solution to form a suspension; 0.2 ml of the suspension is given to the anesthetized hamster by inserting a blunt 19-gauge needle through the vocal cord and stopping just above the bifurcation of the trachea.

The material is injected into the animal, hopefully during inspiration, so that you get dispersion throughout the respiratory tract.

If I can have the first slide, please. (Slide #37.)



Saffiotti, U. Inhalation Carcinogensis
AEC Symposium, 1978

Incidence of tumors in hamsters following the intratracheal instillation of B-a-P.

37

This is the result of one such study. This is from Saffiotti's original work. As you can see, the three dose levels were obtained with 5, 10 or 15 intertracheal instillations with a dose of 3 milligrams BP mixed with 3 milligrams of ferric oxide/installation.

At the low dose, as you see, a total dose of 15 milligrams was administered. This was obtained by 3 milligrams per week times 5 weeks. At that dose level he found a 15 percent incidence of lung lesions with a fairly long latency period or mean latency period of 87 weeks.

At the middle dose, 30 milligrams (again 3 milligrams per week times 10 weeks) gave a much higher incidence. I believe that was approximately 38 percent incidence and the latency period decreased to 69 weeks.

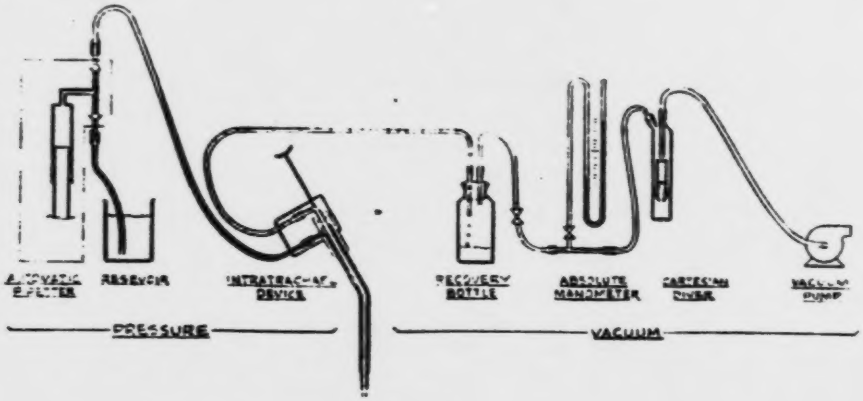
At the higher dose, 45 milligrams, again a much higher tumor incidence was found; in this case, 76 percent and a fairly short (38-week) latency period.

As you can see, this is a very apparent dose-tumor response and this is what we think makes the Saffiotti technique very appropriate for evaluating diesel fuel extracts and/or particles.

The tumors are located primarily in the bronchi and trachea with a few tumors being formed throughout the lung periphery. The most common type is squamous cell carcinoma of the lung. Of course, it also makes it a good model because bronchogenic carcinoma or squamous cell carcinoma of the primary bronchi is the major type of human lung cancer.

We're running short on time they tell me so I will try to pick up. I hope we do not go too fast, but if there are any questions, or if I am going too fast, please slow me down.

The second method (the next slide, #38) is the localized technique, and I'd like to spend quite a bit of time describing this procedure, since it is a fairly new one. Not very much has been published with it but we find that after using it for 3 years at IITRI, we think it is a very good technique.



38

Initially, the technique was developed by Schrieber and Nettesheim at Oak Ridge and it has been modified by them and also by us. I will be describing our particular technique.

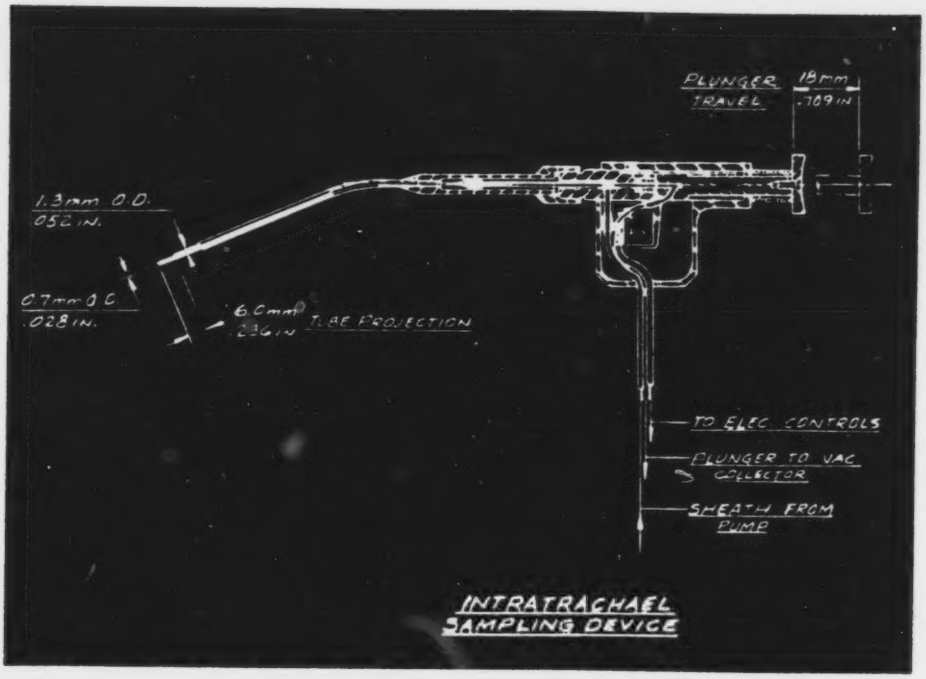
With this method, the test material is placed directly in contact with a very small area of the trachea. Shown is a diagram of the apparatus. The intratracheal device or catheter is shown in the center. The reservoir containing the carcinogen solution is shown at the left. The material is pumped to the catheter and goes into the trachea of the animal. Reabsorption occurs with the equipment shown on the right; the vacuum pump, Cartesian diver (to regulate the amount of vacuum), a monometer (to measure this vacuum).

We found that approximately 14 to 15 millimeters of mercury was enough to ensure reabsorption without doing damage to the lungs. Shown also is the recovery vessel for the nonabsorbed carcinogen solution. (Slide #39.)

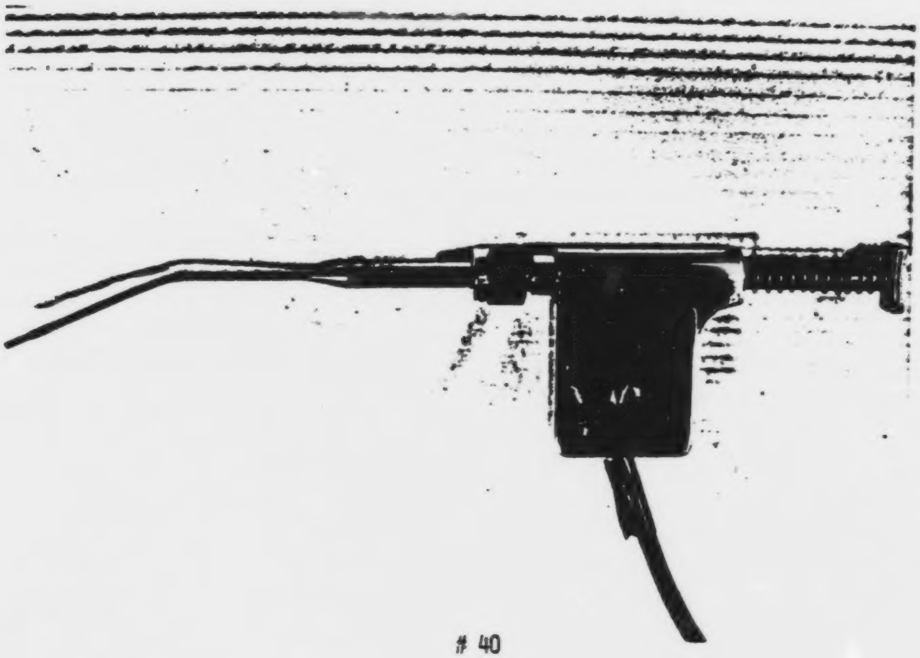
This shows a blueprint of the actual catheter gun itself. I call your attention particularly to the extreme right. As the plunger is pushed down, this extends the tube, as shown on the left, approximately 6 millimeters. There is the tube made of polyethylene tube which is projected out. The outer tube is made of stainless steel. The entire apparatus is also made of stainless steel to give us better stability. The next slide might show this better. (Slide #40.)

This is the actual catheter itself. We've designed the outer tip to fit the trachea of a 7-week old animal. This prevents us from having to house large numbers of animals for a long period of time. For example, the Saffiotti technique requires housing until they're 10 to 12 weeks of age. The most distal tube is the one which technically goes into the trachea; the inner tube projects out to bathe the epithelial cells lining the trachea. (Slide #41.)

This is the apparatus itself in its entirety. To the left, is the Filomatic pump. At the right, the timer box, to let us know how long to stay inside the trachea. We found that 5 seconds is the maximum time that we can stay in the trachea without interfering with the animal's breathing. The next slide shows the actual equipment in operation. (Slide #42.)


PLUNGER TRAVEL
18mm
.709 IN
1.3mm O.D.
.052 IN.
0.7mm O.C.
.028 IN.
6.0mm
.236 IN
TUBE PROJECTION
TO ELEC CONTROLS
PLUNGER TO VAC COLLECTOR
SHEATH FROM PUMP
INTRATRACHAEL SAMPLING DEVICE

39



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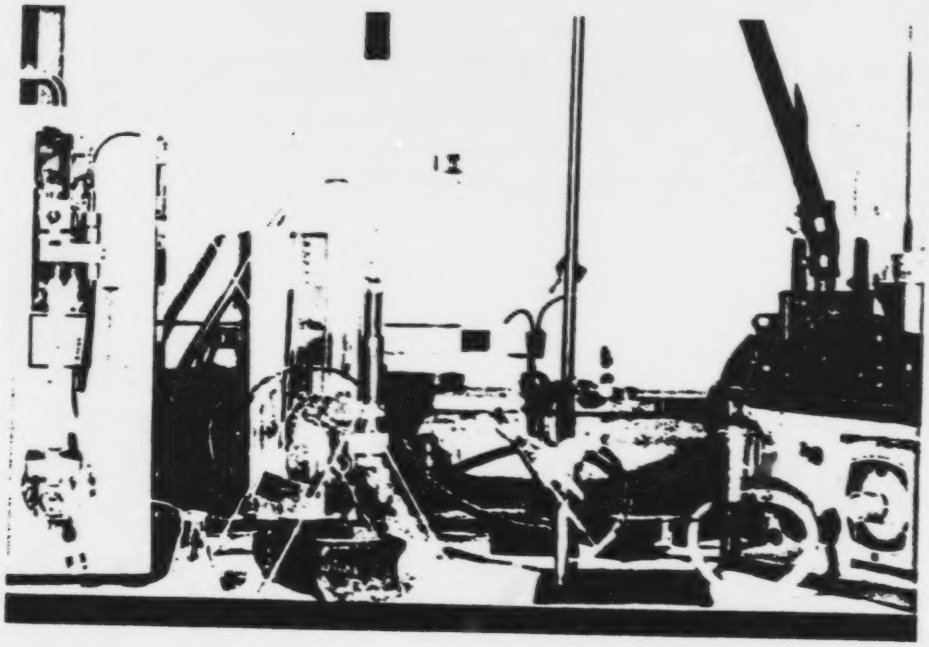

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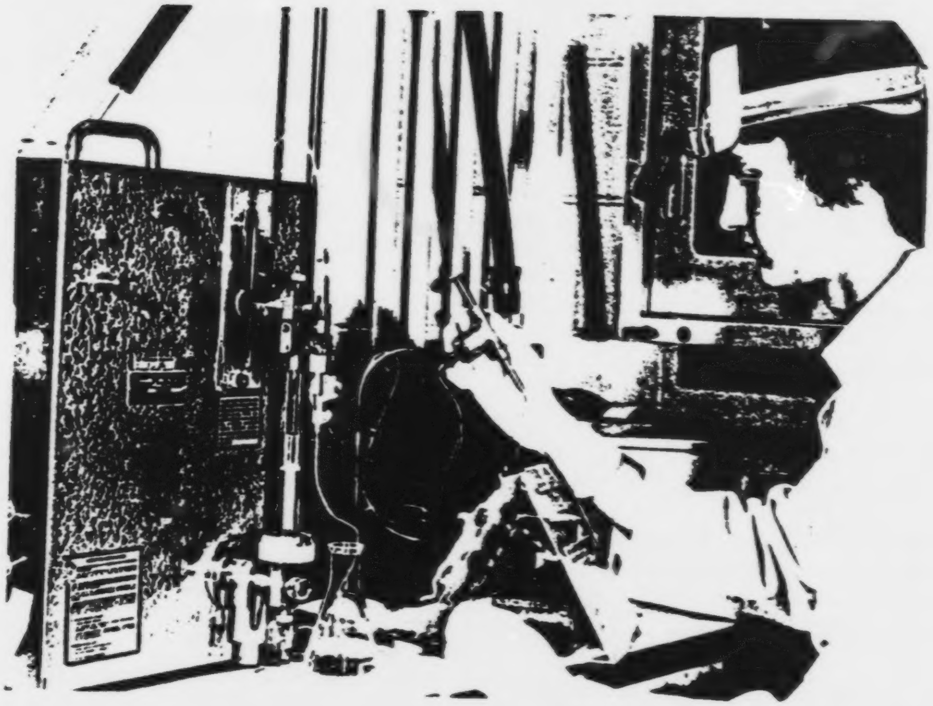
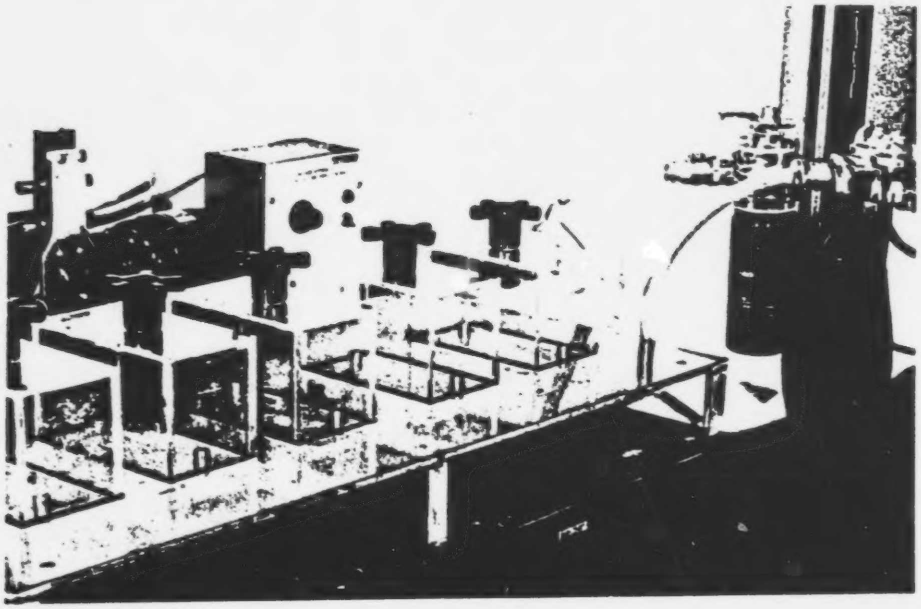


42

As you can see, the hamster is placed on a slanting board. With the use of a headlight mounted on the technician's head, we can actually view the vocal cords. We use a pari of forceps to hold open the epiglottis. As the vocal cords open, we insert through the vocal cords into the trachea. (Slide #43.)



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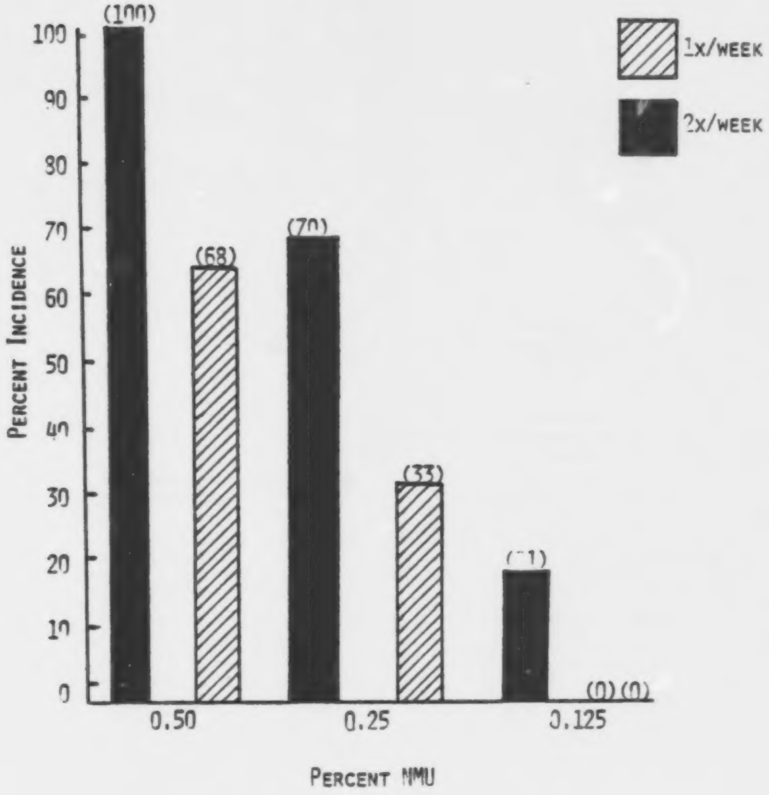
As far as anesthesia, the animals are anesthetized during the process. We've modified the equipment originally developed by Newberne. As you can see, there are individual chambers for anesthetizing the animals. We can keep very close control on how deep the animals are so that we don't lose animals due to anesthesia.

We do use Halothane to immobilize the animals. The procedure takes roughly 30 seconds. Within 1 to 2 minutes after the injection, the animals are awake. (Slide #44.)

This slide shows some of our results. In this case, to the far left, we show the percent incidence and I should point out these are cancers; these are not benign tumors, and I'll emphasize this a little bit later on.

In this case, animals were treated either once a week, shown in the slash lines, or twice a week, shown in the solids, for 15 weeks with either 0.5 percent NMU or methylnitrosourea; 0.25 percent or 0.125 percent. Treating animals two times a week, to the far left, gave us 100 percent incidence of tracheal squamous cell carcinomas within 6 months.



EFFECTS OF N-NITROSOMETHYLUREA (NMU) CONCENTRATION AND NUMBER OF INSTILLATIONS ON TRACHAEL CANCER INCIDENCE

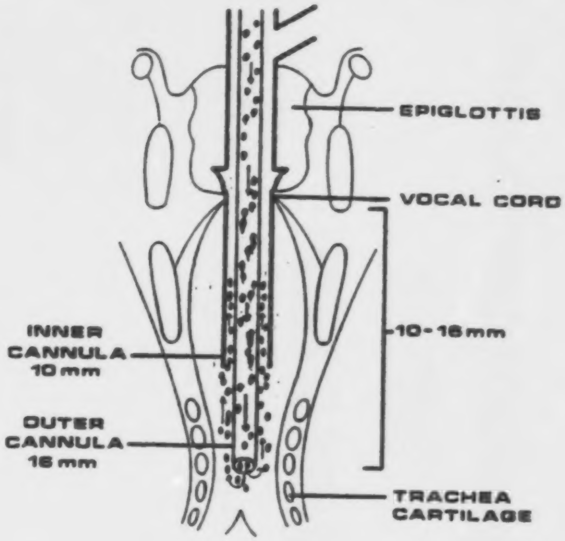
TRACHEAL CANCER INCIDENCE 6 MONTHS AFTER THE INITIAL INTRATRACHEAL INSTILLATION OF NMU.

44

At the 0.25 percent level, we had a 70 percent incidence of cancer, and the 0.125 percent level, 21 percent. Very good dose-tumor response. Also treating one time a week, the slash lines at the high NMU dose of 0.5 percent, a 68 percent cancer incidence; middle dose, 33 percent incidence, and the 0.125 or lowest level, we were not able to detect any cancers at 6 months after the initial instillation. (Slide #45.)

To back up just a little before getting to the pathology of these tumors. This is a drawing of how the trachea is bathed with the material. The inner catheter and outer catheter are actually reversed on the drawing. We won't go into that now.

INTRATRACHEAL INSTILLATION MODEL FOR CARCINOGEN SCREENING



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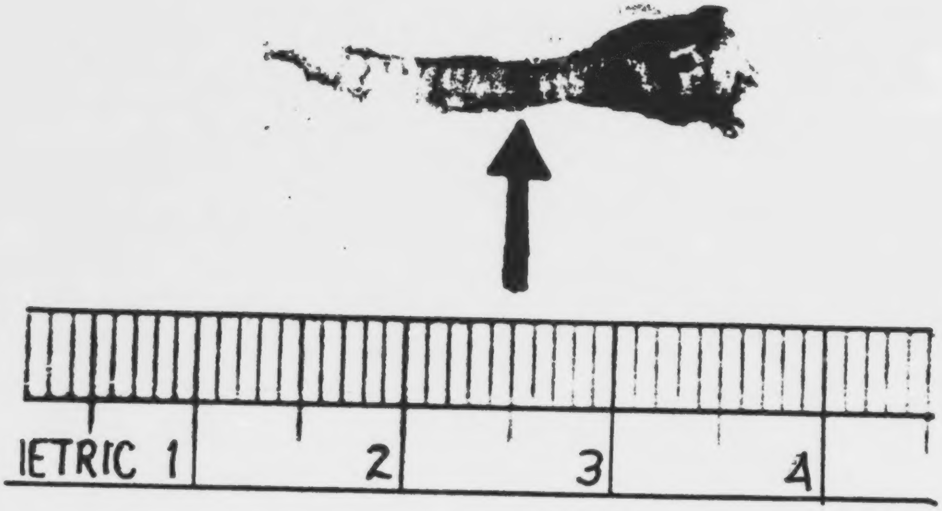
The material flows down at the black line which is where it ends. This is where the fluid comes in contact with the tracheal epithelium. It flows down to the end of the yellow tube shown here where it's reabsorbed. Again, a 5 to 6 millimeter area of exposure. (Slide #46.)

This slide shows how these tumors appear grossly. This is taken from an animal that received a half-percent NMU, one time a week for 15 weeks. At roughly 5 months the animal developed respiratory trouble and as you can see, the tumor is localized approximately in the midpoint of the trachea. (Slide #47.)

Another shot showing how extensive these cancers can become, almost occluding or at least blocking out any gross view of the tracheal cartilege. (Slide #48.)

A little bit on histology. This is a control or solvent-treated animal at 6 months, showing a fairly normal epithelium. He has a normal pseudostratified columnar epithelium. The technique itself, using the solvent, does not appear to do any damage. (Slide #49.)



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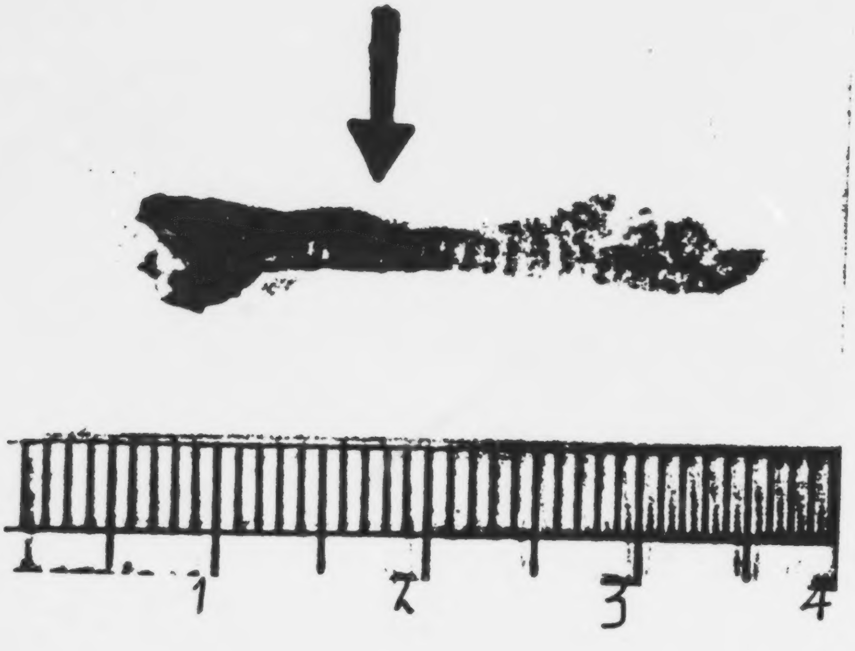

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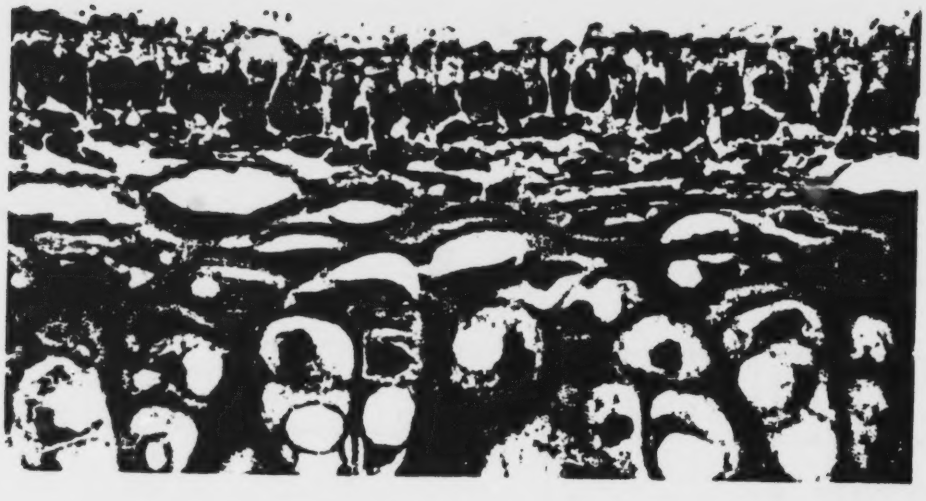

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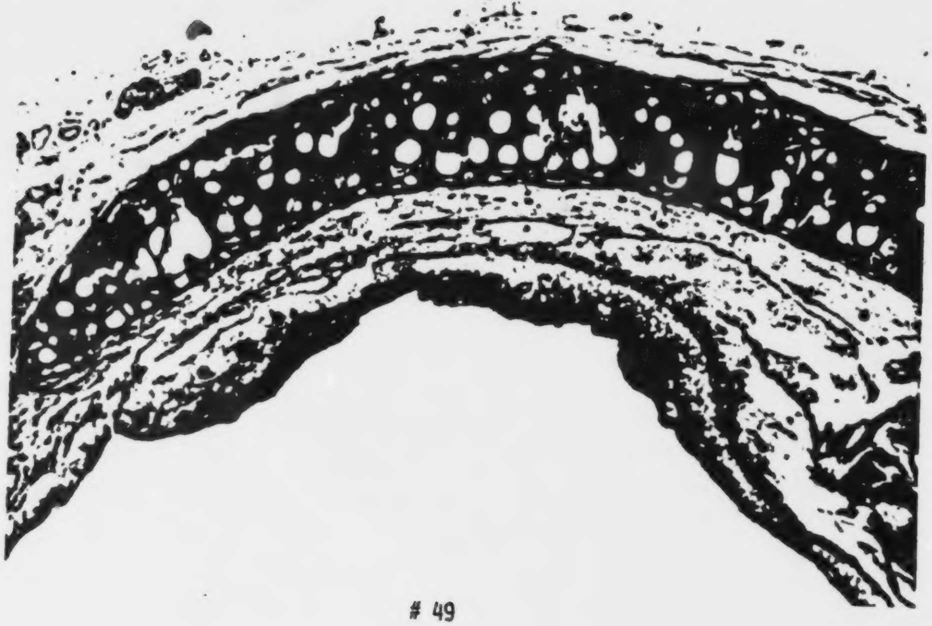


49

This shows that not all the lesions that we induced were actually cancers. In this case this was diagnosed as squamous cell metaplasia or squamous metaplasia. (Slide #50.)

This shows how extensive this can become. It shows an extreme cornification or keratinization of the cells. Getting back to the next slide--(Slide #51.)

Getting back, we said the data I showed you earlier was with cancers. This is how the cancers appeared histologically, both invasion out into the trachea, or into the lumen of the trachea, and also invasion into the submucosa or lamina propria. (Slide #52.)

View showing extreme keratinization. Extremely characteristic of squamous cell carcinomas. I might mention that we've also recently done some studies with PAS alcium blue to see if there is an adeno component to these tumors and indeed we do find that many of the tumors also show adenomatous component.

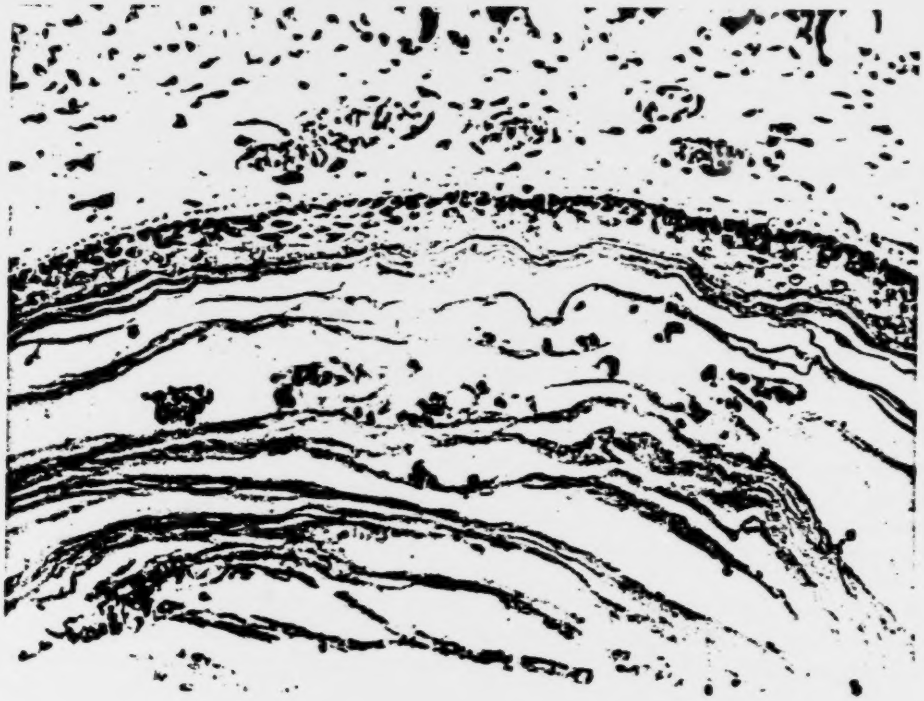
This agrees with some of the work by Trump and McDowell at the University of Maryland in which human bronchogenic carcinomas are now thought to be both or combined epidermoid and adenocarcinomas (Slide #53.)

This shows how extensive the tumors can appear and are, in some cases, actually the [illegible] of death in these animals. It does eventually completel[illegible]ck the lumen of the trachea. (Slide #54.)

To show you that we've also begun work with different types of chemical carcinogens using this system. In this case we used the compound nitrosoethylurea. That is, we replaced the methyl group with an ethyl group. We administered the compound once a week for 15 weeks. In this case, we weren't sure what the best solvents were, so we used two: one, acidified saline; the other, ethanol and water. In both cases we had roughly an 80 to 83 percent incidence of tracheal tumors at 6 months.

I emphasize the word tumor. These tumors were not cancers. They were papillomatous in structure or in pattern, projecting out into the lumen of the trachea. We found no evidence of invasion and, therefore, we did not classify them as carcinomas.

We found it most interesting that changing one group on a chemical gave us a very diverse or different type of tumors. From the structure-activity relationship (or for those type studies) we feel like this particular technique will be of benefit and also we feel like it's going to be good for identifying various types of possibly carcinogenic agents found in diesel fuel extracts. (Slide #55.)



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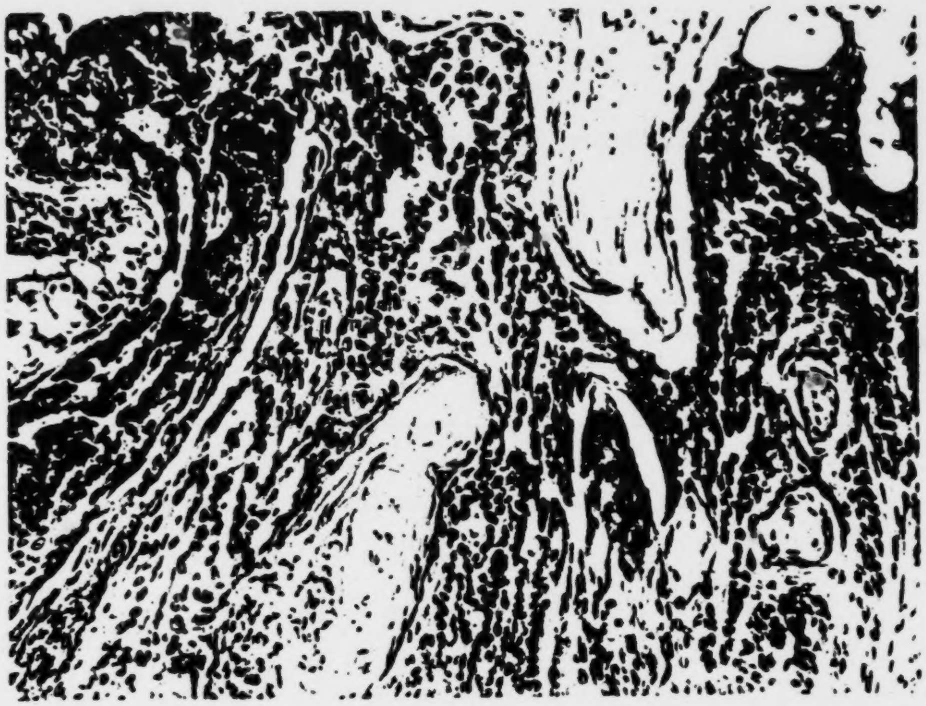

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EFFECT OF N-NITROSOETHYLUREA (ENU) ON THE TRACHEAL EPITHELIUM OF HAMSTERS

	INITIAL NO. OF ANIMALS	EFFECTIVE NO. OF ANIMALS	NO. OF ANIMALS WITH TRACHEAL TUMORS (%)
ENU 0.5% IN ACIDIFIED SALINE	20	16	13(81)
ENU 0.5% IN ETOH:H_2O 10:90	20	12	10(83)

INCIDENCE OF TRACHEAL TUMORS INDUCED WITH ETHYLNITROSOUREA.

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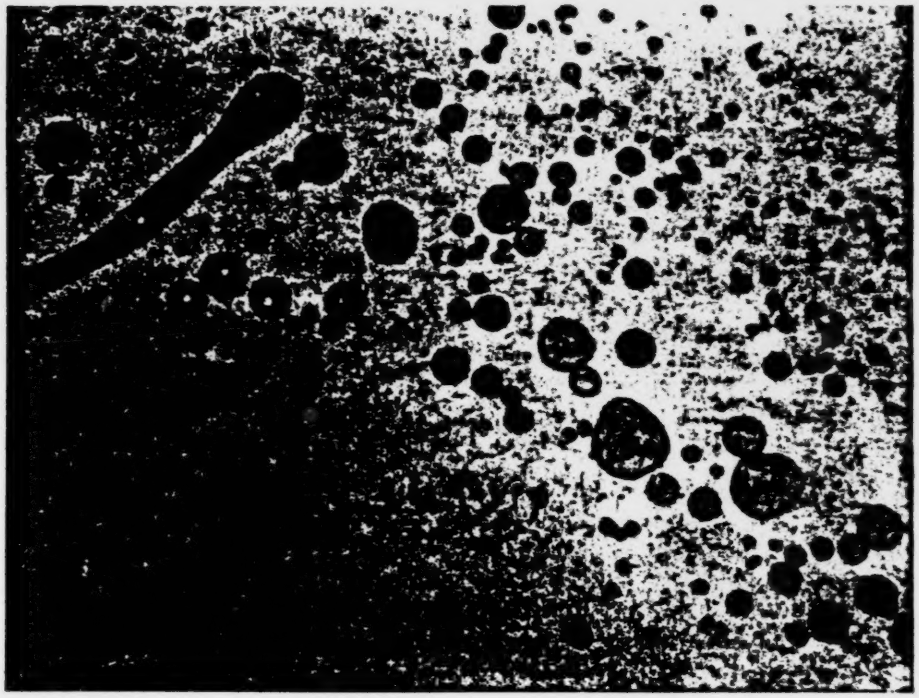

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I'd like now to present some of the preliminary work that we have started at IITRI. I realize, time-wise, we're running short. We will try to go fairly quickly but basically, our problem initially was to find a suitable solvent. EPA supplied us the material dissolved in methylene chloride. Obviously we couldn't administer this to animals with either the Saffiotti or the Schreiber (or localized) technique.

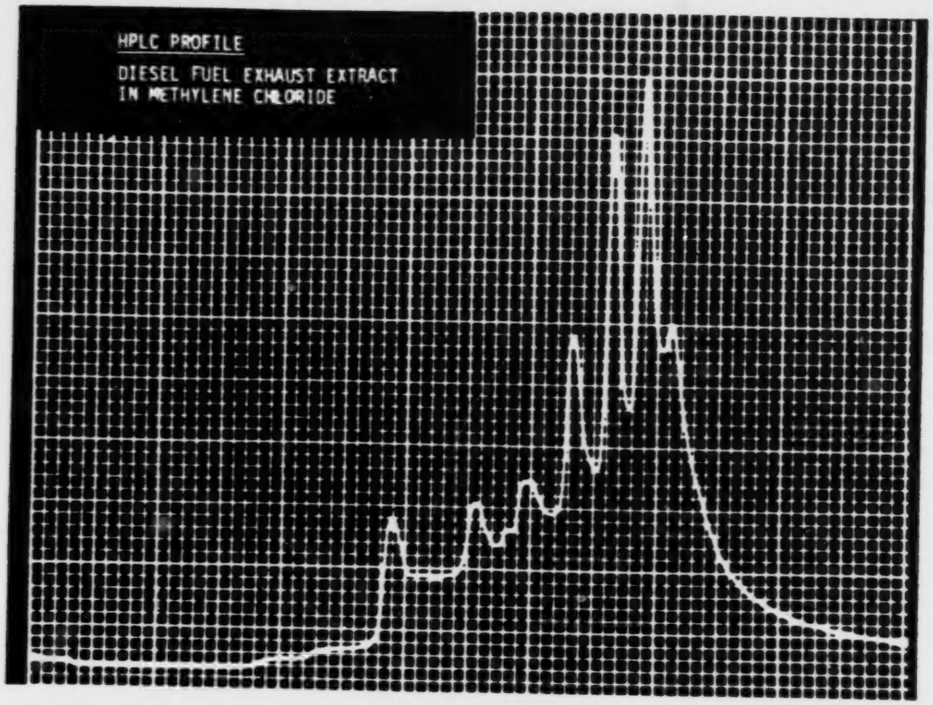
So because of this we began several studies to find the best solvent that would keep the material in solution rather than precipitating out. So before we started our manipulations we wanted to see what the material looked like at the time that it came to us.

This is a photomicrograph of the methylene chloride extract of diesel fuel exhaust extract. You can see that there are droplets of oil, very light oil. Roy Zweidinger and I have discussed this and we feel like it may be paraffins which the methylene chloride has washed off or it may have come from the filter itself. But this is what we wanted to try to reproduce in our studies with the animals. (Slide #56.)

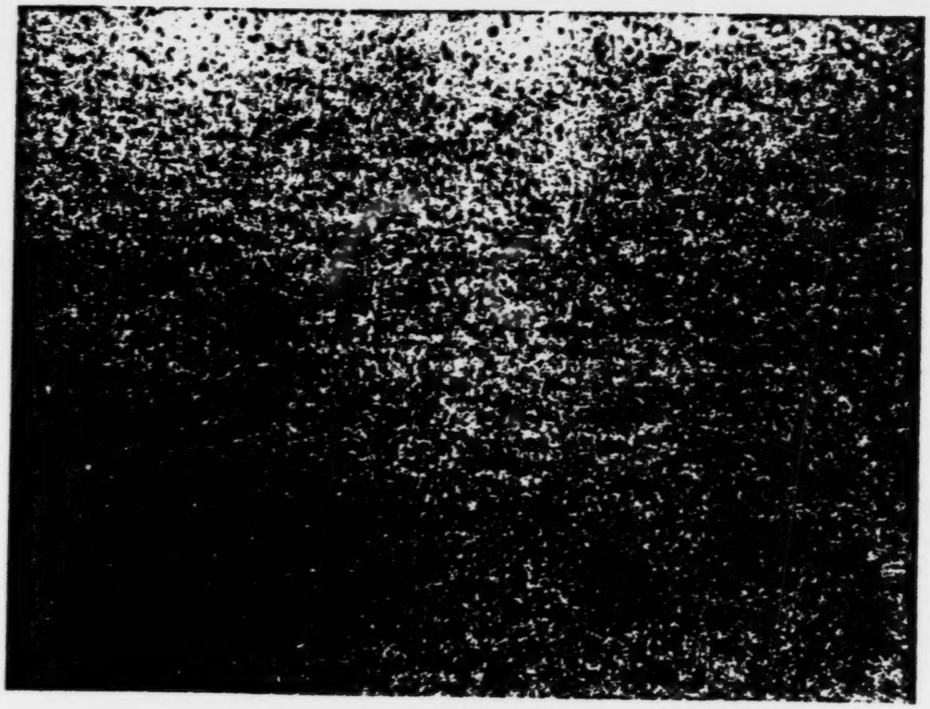
We also wanted to characterize it with HPLC. This is the reverse phase of HLPC work using a particular setup, which I won't go into detail, at IITRI. We found that there were seven major peaks with several minor peaks. This is what we wanted to confirm; that whatever manipulations we made with different solvents, we still maintained this particular pattern. (Slide #57.)

This is a photomicrograph of the material in pure DMSO. DMSO, of course, is a very good solvent for diesel fuel exhaust extracts. Unfortunately, our equipment will not let us administer pure DMSO, partly because of viscosity and partly because we didn't know what the toxicity of administering large quantities of pure DMSO would be in an animal. But this is what it looks like in pure DMSO. (Slide #58.)

HPLC pattern to show again that DMSO did not affect it. (Slide #59.)


HPLC PROFILE
DIESEL FUEL EXHAUST EXTRACT
IN METHYLENE CHLORIDE

56



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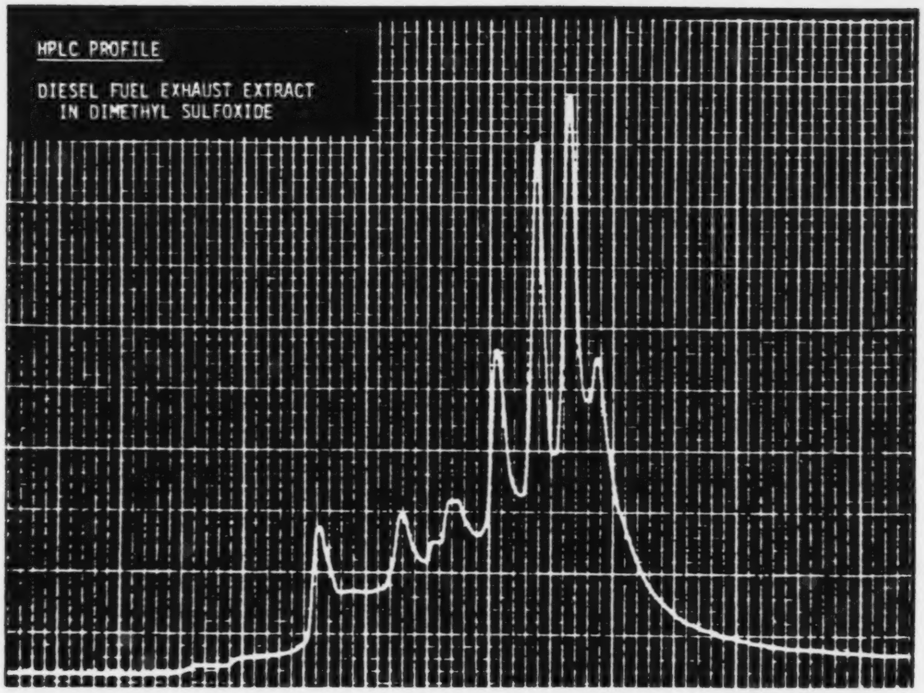
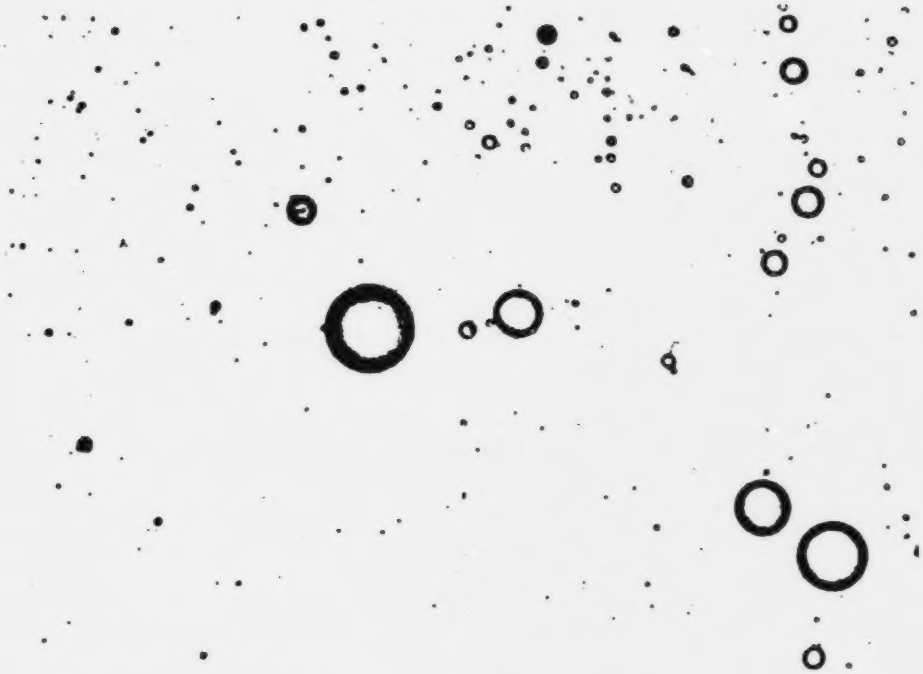

HPLC PROFILE
DIESEL FUEL EXHAUST EXTRACT
IN DIMETHYL SULFOXIDE

58



59

Our first attempts were to try to find out if we could use DMSO in a saline solution. We did a preliminary study at IITRI in administering concentrations of DMSO up to 25 percent. We did that for 10 weeks. We found no toxic effects on the cells so in our first study was to try 25 percent DMSO, 75 percent water or saline. In this case, the material looks fairly good. You do find the light oil droplets but we also found, as you can see, some dark material, more or less coating the light oil. (Slide #60.)

Knowing this (until we found a better solvent) we wanted to get animals on test. This is the protocol that we followed. We're treating all animals one time a week, in this case, for 10 weeks. As you can see, we treated at 1 percent, 0.5 and 0.25 percent dose levels. We tried a 1.5 percent solution, 2 percent solution and so forth, but we found that during the instillation procedure the material precipitated out so 1 percent was the maximum we could inject. (Slide #61.)

Because of the light oil we thought we'd try adding an ethanol component to our solvent system to try to dissolve or get the light oil droplets into solution. Ethanol itself did not work, but we added to the ethanol and DMSO mixture, propylene glycol, which is another solvent for oils, supposedly nontoxic. We ended up with a four-phase system this time--10 percent DMSO, 10 percent ethanol, 20 percent propylene glycol, and 60 percent saline.

This looked good to the naked eye. It didn't precipitate out but when we viewed it with high microscopy, we did find very, very small dark oil droplets. Again, we preferred not having those. (Slide #62.)

This particular procedure did not grossly affect the HPLC pattern. The peaks are a little bit lower than in the previous slides but we felt it may be partly due to injection into the HPLC, in other works, a smaller quantity of the material was put into the HPLC. (Slide #63.)

As shown, animals receiving this solvent were placed on test. I forgot to mention. I wanted to give you some data as far as the current status of these protocols.

The first study was for 10 weeks. Last week there were no differences in body weights. They had a small mortality. I think two animals died in the whole study, but they were not related to diesel fuel. So from this study we don't feel that diesel fuel is going to be highly toxic in our system.

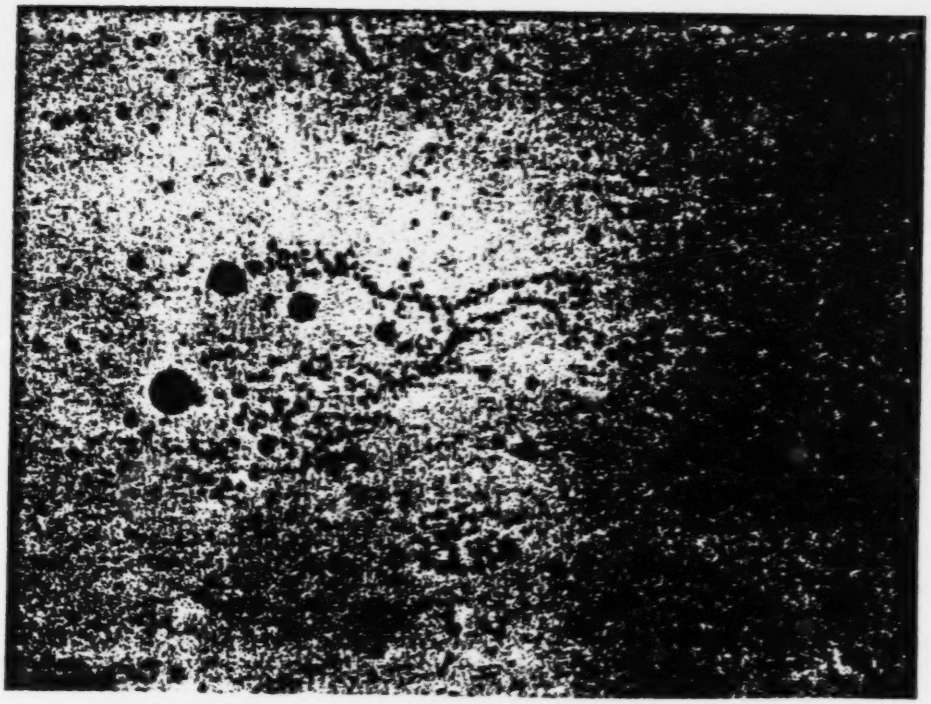
In this case, we're 6 weeks into this study. We've lost no animals. The animals were still gaining weight comparable to controls, so there's no problem.

Protocol for Dose-Range Study to Determine Toxicity of Diesel Fuel Extract Using the Localized Intratracheal Instillation Technique at IIT Research Institute

Group	No. of Hamsters	Treatment
1	25	Solvent (25% DMSO)
2	25	1.00% Diesel Fuel Extract
3	25	0.50% Diesel Fuel Extract
4	25	0.25% Diesel Fuel Extract
5	10	Shelf Control

Protocol for treating hamsters with the localized technique (diesel fuel exhaust extract in 25% DMSO, 75% saline).

60



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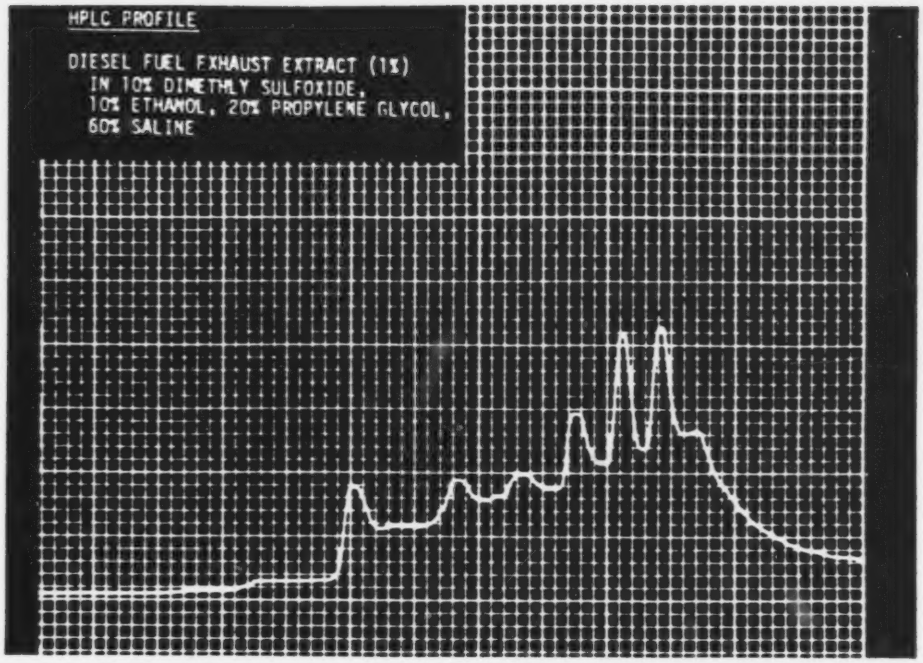

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Protocol for Dose-Range Study to Determine Toxicity of Diesel Fuel Extract Using the Localized Intratracheal Instillation Technique at IIT Research Institute

Group	No. of Hamsters	Treatment
1	25	Solvent (10% DMSO, 10% ETOH, 20% Propylene glycol, 60% Saline)
2	50	1.00% Diesel Fuel Extract
3	50	0.50% Diesel Fuel Extract
4	50	0.25% Diesel Fuel Extract
5	10	Saline
6	10	Shelf Control

Protocol for treating hamsters with the localized technique (diesel fuel exhaust extract in 10% DMSO, 10% ETOH, 20% propylene glycol, 60% saline).

63

You might notice that we've also included a saline group just to see if our solvent system does have any toxic effects. (Slide #64.)

The best preparation we found so far is this one; a 10 percent DMSO, 20 percent propylene glycol and 70 percent saline. Why the ethanol made our preparation worse we don't know, but when we went back and did the 20 percent propylene glycol without ethanol, we found this microscopic pattern.

This again is the 1 percent solution of diesel fuel extract. I should have mentioned, all the photomicrographs I've shown were of a 1 percent solution. (Slide #65.)

In this case, the comparison is made to a methylene chloride solvent, the material as it came in to us. To me, it looks better than what we received, so we feel like this is going to be a good solvent for administering diesel fuel extracts. (Slide #66.)

Again the HPLC pattern in this particular solvent; very similar to methylene chloride or the original material. (Slide #67.)

This is a the animal study we have going using this solvent; this is in localized technique; the same protocol, 1 percent, 0.5 percent, 0.25 percent again, using saline controls to check out any toxicity of the solvent. (Slide #68.)

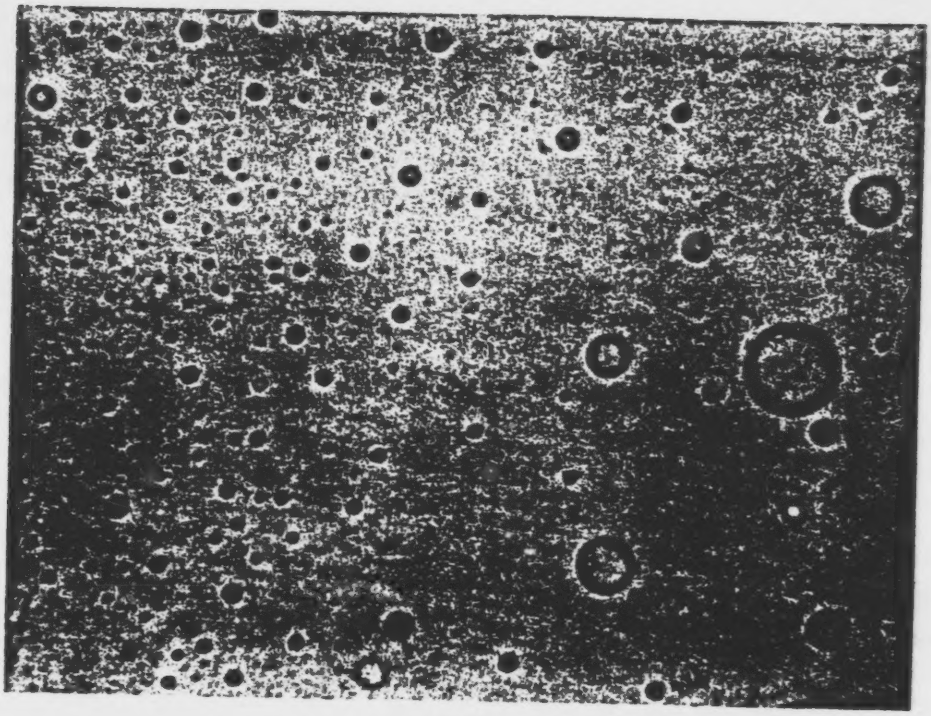
Now, in addition, while we had the localized technique studies going, we also wanted to try to get something started using the Saffiotti method. This is two protocols which we have in progress now.

The first study we started with the Saffiotti method had our 10, 10, 20, 60 solvent system. That was the best we had at the time it started. We're 5 weeks into the study. There's no indication of any toxicity in any of the top three groups. I should point this out. The animals are being treated one time a week. We plan to go for 15 weeks.

As you can see, a 1 percent solution administered 0.2 ml of the solution gives you approximately, not approximately, but gives you actually 2 milligrams of material into the lungs. We were looking for toxicity. We decided to try doubling the amount of material. We couldn't go up in percent because the material precipitated out but with the Saffioti method we could administer it twice a week.

This is the basis of the last three groups, Groups 4, 5, and 6. They were treated twice a week. That means the animals in Group 5, for example, were receiving 4 milligrams of material per wek.

We have lost one animal in Group 5 and we've lost one animal in Group 4. So we really can't say anything about toxicity. (Slide #69.)



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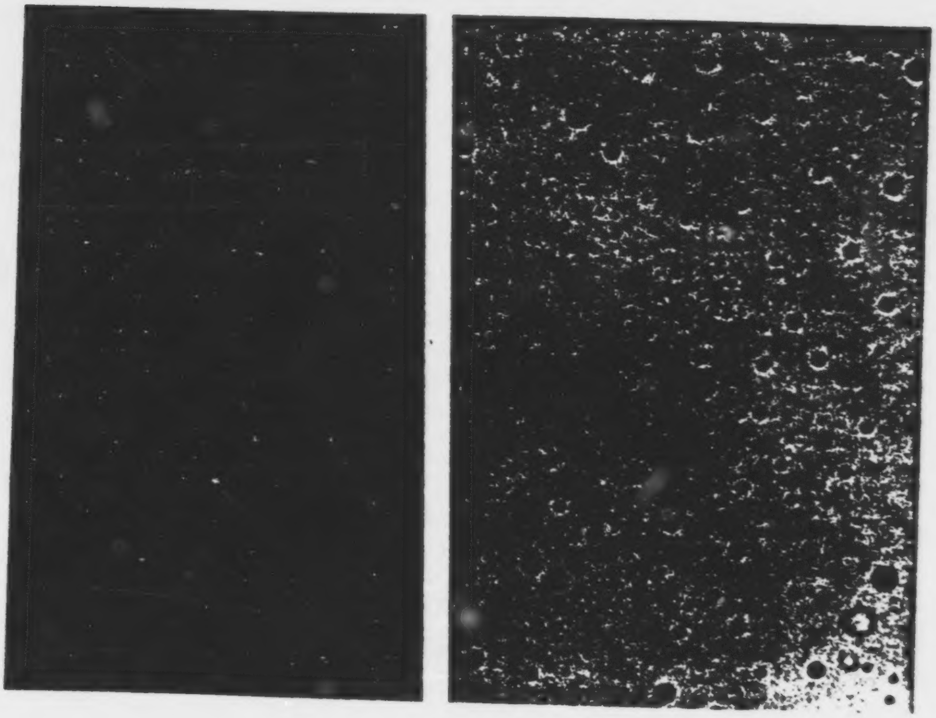

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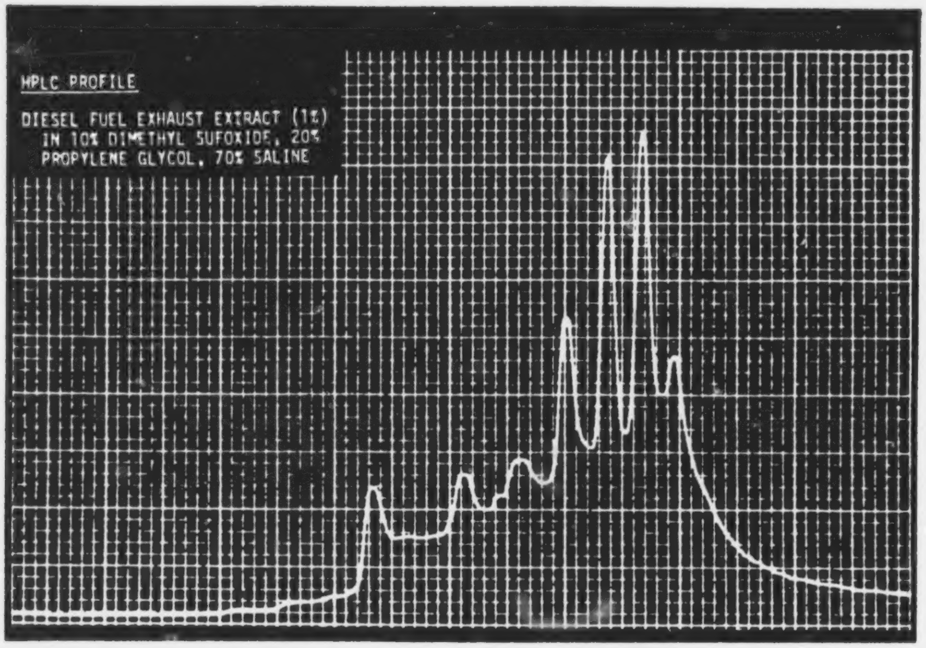

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PROTOCOL FOR DOSE-RANGE STUDY TO DETERMINE TOXICITY OF DIESEL FUEL EXTRACT USING THE LOCALIZED INTRATRACHEAL INSTILLATION TECHNIQUE AT IIT RESEARCH INSTITUTE

GROUP	NO. OF HAMSTERS	TREATMENT
1	25	SOLVENT (10% DMSO, 20% PROPYLENE GLYCOL 70% SALINE)
2	50	1.00% DIESEL FUEL EXTRACT
3	50	0.50% DIESEL FUEL EXTRACT
4	50	0.25% DIESEL FUEL EXTRACT
5	10	SALINE
6	10	SHELF CONTROL

PROTOCOL FOR TREATING HAMSTERS WITH THE LOCALIZED TECHNIQUE (DIESEL FUEL EXHAUST EXTRACT IN 10% DMSO, 20% PROPYLENE GLYCOL, 70% SALINE).

67

Protocol for Dose-Range Study to Determine Toxicity of Diesel Fuel Extract Using the Saffiotti Intratracheal Instillation Technique at IIT Research Institute

Group	No. of Hamsters	Treatment
1	20	Solvent (1X/week)
2	20	1.0%, 2mg/Instillation (1X/week)
3	20	0.5%, 1mg/Instillation (1X/week)
4	20	Solvent (2X/week)
5	20	1.0%, 2mg/Instillation (2X/week)
6	20	0.5%, 1mg/Instillation (2X/week)
7	10	Shelf Control

Solvent - 10% DMSO, 10% ETOH, 20% Propylene Glycol, 60% Saline

Protocol for treating hamsters with the Saffiotti technique (diesel fuel exhaust extract in 10% DMSO, 10% ETOH, 20% propylene glycol, 60% saline).

68

Protocol for Dose-Range Study to Determine Toxicity of Diesel Fuel Extract Using the Saffiotti Intratracheal Instillation Technique At IIT Research Institute

Group	No. of Hamsters	Treatment
1	15	Solvent (10% DMSO, 20% Propylene glycol 70% Saline) -- 2X week
2	20	1.0%, 2mg/Instillation -- 2X/week
3	20	0.5%, 1mg/Instillation -- 2X/week
4	15	Solvent (20% DMSO, 20% Propylene glycol, 60% Saline) -- 2X/week
5	20	2.0%, 4mg/Instillation --2X/week
6	20	1.0%, 2mg/Instillation -- 2X/week
7	10	Shelf Control

Protocol for treating hamsters with the Saffiotti technique (diesel fuel exhaust extract in 10% DMSO, 20% propylene glycol, 70% saline; and in 20% DMSO, 20% propylene glycol, 60% saline).

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We also have a study in progress using the solvent system, 10, 20, and 70. In this case we're again trying to find some toxicity. We're treating two times a week. To go higher up to see if we can find some data which might cause changes in the animals, we changed the solvent system in Group 4 to 20 percent DMSO, 20 percent propylene glycol and 60 percent saline.

By doing so we were able to get a 2 percent solution that didn't precipitate out during the instillation procedure. This would mean we are giving, and I'll show you a photograph of that in a minute, but we are giving 8 milligrams per week. We have lost two animals in Group 5 after 4 weeks on test. The animals are showing a failure failure to gain weight. (Slide #70.)

As I said, this is the 2 percent solution. As you can see, it does not precipitate out grossly but we have that very heavy oily material, which means something is coming out of solution. (Slide #71.)

We have done a few characterization studies on the particles themselves. I show this slide to show what diesel fuel particles look like. In this case this is the actual material that came in to us. The particles are rather large. The one in the center, for example, is roughly 150 microns but if you look closely it appears that these particles are coalescing or actually sticking together.

I think someone mentioned earlier today that normally the particles are less than 2 microns. So we haven't done any work as far as trying to break these up yet, but we will be trying techniques such as grinding in a mortar or perhaps ball-milling or some other technique to make sure that we can get these particles, hopefully, back to the shape that they were in when they originally came out of the engine or at least get them down to less than a 5 micron diameter. (Slide #72.)

This is a high power view of the same slide. We estimate that 70 to 80 percent of the diesel particles that we have, as it comes to us, is less than 5 microns. The other particles, probably 99 percent, will be less than 20 microns. We don't feel that we have a real problem as far as getting these back to, or less than, 5 microns in size. (Slide #73.)

The last slide is just to show you what we hope to get with diesel fuel particles. This is an actual preparation of benzo-a-pyrene and ferric oxide, using polarized light.

The light areas that you see up there is the benzo-a-pyrene. The dark ones are ferric oxide. This is what we would hope to be able to attach the diesel fuel particles to in at least one of the groups and attain some type of association as shown here.

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I guess in conclusion, since my time has run out, I'd just like to say we feel that sufficient data will be obtained using both of these techniques to determine if, and I want to emphasize this, if topically applied diesel fuel particles and extracts do have a carcinogenic effect on respiratory epithelium. In essence, what we're saying and what we hope is that each of the techniques will confirm and reinforce the findings of the other one.

Thank you.

DR. FLAMM: I think one of the things that we need to ask is to what extent is the modified Saffiotti method likely to provide information that the Schreiber method itself won't provide? Could you speak to that?

DR. GRUBBS: Basically, we hope that both procedures will provide the information for us. The primary reason for the localized method is the time factor. In other words, I hopefully convinced you that with known carcinogens we can get a very high incidence of cancers within 6 months. We can use this for comparison of the diesel fuel particles to see if indeed they are as carcinogenic as a known carcinogen, such as methylnitrosourea. Failing that, at least we can compare it to the control animals. We can also find out if the materials in diesel fuel extract produce rapid changes in the epithelial cells themselves.

This particular device I mentioned is one that can be used for tracheal cytology. So we can do tracheal cytology and evaluate very quickly the response to these particular solvents.

MR. SHEFNER: A good deal of the time at this meeting was spent on what will be known by August to October of 1979. One of the values of this technique, the modified Schreiber, is the fact that we expect to have results by that period of time. We won't have them with the other.

DR. ALBERT: Isn't it so that you haven't induced tracheal cancers with insoluble carcinogens?

MR. SHEFNER: These are not insoluble.

DR. ALBERT: They're not?

MR. SHEFNER: You saw the slides. What you saw primarily was oil droplets in this mixed solvent. Either they are in solution or in such fine suspension that they will go through. They're not insoluble as you refer to them.

In fact, that's why I said in my introduction that I thought it was a model technique. If you want to do comparison studies with the other collected particulates or emissions, you can make parallel DCM extracts of those three that were talked about, coke oven emissions, roofing tar and cigarette smoke condensate, bring them into solution in the solvent which can be used for treatment and get your comparison study for the matrix by the 1979 date if we were authorized to start them.

DR. FLAMM: What is unclear to me is the basis for the modified technique providing results at earlier time than the Saffiotti procedure. That's what just isn't clear at all and, you know, the work that you showed was with very powerful carcinogens where it's possible to get tumors in 8 weeks in certain systems for that matter. So 6 months is not all that impressive with those kinds of carcinogens.

MR. SHEFNER: I don't think we can give you an answer in the sense of can we say that we know that we will get a response to this material in a shorter period of time. The suggestion was that with this technology and the limited area to which you have exposure, you have at least less investment in seeing whether you're going to get a response because you have, as Dr. Grubbs showed, a very nice fine localized area of application and in fact the response occurs only there.

It's a lot easier to survey that than to go through a whole Saffiotti procedure with this material in the particular period of time.

DR. FLAMM: Well, I think the other thing that troubles me is whether this kind of information is going to be very useful in terms of looking at comparative or relative potency. I mean, obviously, when you're painting the animals' backs, we have a range, whereas now we're just hitting a specific spot in the trachea. You either do or do not have a tumor at that point and whatever range you may be able to generate would have to be dependent on the frequency or incidence within the population group that you're using which is very small or based on differing concentrations.

MR. SHEFNER: I don't see the parallel. If you paint the animal's back you certainly know what you apply but you don't know what was absorbed. So you really don't know what dose the animal was given. In all skin paintings then you can say I applied so much material to the back of the animal. We're saying I applied so much to the trachea.

Now, in terms of range of response, if you're talking about primarily skin painting, the response is measured in terms of pathology. You can go from papillomas to defined cancer and numbers of each.

DR. FLAMM: No, that is not quite right.

MR. SHEFNER: What range of response are you talking about?

DR. FLAMM: I'm talking about a quantitative range of response because this is somehow supposed to relate not to the question as to whether there are carcinogens there; we know that that is likely to be the case. We are trying to use data in a comparative way to get some feel for relative potency and it's not clear to me that the modified technique lends itself particularly well to that subject.

MR. SHEFNER: I think if you're only going to compare it to the MNU control then you may well be right in that if you have a highly potent carcinogen as control and I would not expect to see as large a response as with MNU. If in fact you use the other materials for which you have epidemiological knowledge, then if nothing else, you can say one substance appears to be similar to, or more or less potent than another by the degree of histopathological change, which is the way most such studies are evaluated, numbers and degree.

DR. GRUBBS: I might just add that with the Saffiotti technique there is a tremendous variation from laboratories to laboratories and even within individual laboratories as far as dose tumor response. This is partly a problem because of carrier dust size. There are also problems of how much the technician is injecting. All kinds of problems have created a variation from one lab to the other with Saffiotti's technique.

With the Schreiber or localized technique, granted we've done it only at IITRI. I have to take that back. Dr. Nettesheim at Oak Ridge is continuing to perform studies with this technique and he is finding that this is also a very reproducible model. So I think that we do have some evidence that this technique is reproducible and I think it's good evidence for performing such a study with diesel fuel exhaust emissions.

DR. CORTESI: I don't quite understand how this stuff, in and out, you basically get anything into the animal. Now, obviously, I'm missing a point somewhere and perhaps you could address that slightly.

DR. GRUBBS: I think just to refer to the slide where we showed the gross tumor indicates that there is something getting into the tissue.

DR. CORTESI: But there's something pretty powerful there.

DR. GRUBBS: Yes.

DR. CORTESI: But I think maybe 5 seconds, so it's something that's going to be a relatively mild carcinogen. I mean, do you consider it unlikely that you will observe the results with this or not, on these sort of things?

DR. GRUBBS: Maybe I'm missing your point. Do we know that this is a mild carcinogen?

DR. CORTESI: Well, I can't answer that. I'm not willing to stick my neck out.

DR. ZEIGER: I think more to the point, how do the two techniques compare when the same carcinogen, when benzo-a-pyrene, is used? Is the modified technique effective against benzo-a-pyrene or polycyclic hydrocarbons?

MR. SHEFNER: It hasn't been tested yet.

DR. ZEIGER: Okay.

DR. FLAMM: Okay. We'll probably come back to--almost certainly we will come back to this tomorrow morning. We'll have to move ahead now to the next speaker who is Robert Miday, who will be speaking on epidemiologic studies.

DR. MIDAY: I'm going to review epidemiologic studies and I'll be talking both about studies that have been completed and are now in the literature and studies that are underway, some by other government agencies, several foreign studies, and also the investigations that we are planning to do during the upcoming 1 to 2-year period. We do not have specific epidemiologic studies underway at the present time so I will not be able to give you details of study protocols

Epidemiology potentially provides extremely important information because it looks directly at health effects in human populations and obviously this is the bottom line. However, careful interpretation is always very important when looking at the studies. All have limitations and the strength that eventually comes about to establish causation in any particular exposure is most often the result of multiple studies that are able to look at different populations using different study designs.

It's virtually impossible to definitely answer all the important health questions about a particular exposure with one investigation and this holds just as strongly in the area of diesel emissions.

The major difficulty in attempting to get underway studies in this area is that of identifying exposed study populations that can be investigated. The difficulty comes about because of several reasons. There are certain characteristics that are necessary before a group that appears to be exposed is appropriate to and can be looked at from an epidemiologic standpoint for health parameters.

I've put on the first transparency* some of the major factors that always have to be kept in mind when thinking about looking at a specific group.

First of all, there has to be some exposure index that's documented although this often is only a crude index, such as occupation. In this case we might be considering mortality studies, where the only index we would have might be that the person was a driver of a specific type of vehicle or that they were a worker in a specific mine or garage.

It would range widely from such crude estimates to those where you have actual measurements for each individual of a blood level of a toxin, where you can quantitate fairly exactly what exposures are.

Secondly, the size of the population is always crucial and in this area it is very often a limiting factor. Basically, it has to be large enough to detect the health effects with a statistical power that's desired.

In other words, you're saying that the effects that you do detect are not a result of chance and that if you don't detect defects you're able to say with a certain level of confidence that this could not be due to chance.

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* Transparencies unavailable for this report.

The next point is the fact that when you're looking at health parameters, particularly retrospectively, it is necessary to have records available of the health data and this is something that has to be applicable to the entire group or to a very large proportion of the ntire group, for it to be usable.

And lastly, a comparison group always enters into the design of the study. In most of these studies the ideal comparison group would be a similar group exposed to gasoline exhaust emissions, and/or a group who were unexposed to any similar type of toxin. It would be difficult to find gasoline-exposed groups, but it might be possible in some studies.

Now, in spite of all these factors, there have been several occupational populations that have been identified and look promising from an epidemiologic standpoint, and these boil down, in my opinion, to the following. First of all, workers who service diesel engines or workers who work around areas where diesel engines are being serviced in relatively enclosed spaces, such as large garages. These would be workers who were repairing engines on either buses, trucks, trains and possibly ships, and their coworkers who might be doing ancillary duties in the same areas.

There are some good groups here. The problem is there are not many groups who are in this or a large size category. Thus you have to compile several population groups to get numbers that are usable for most of the health parameters of concern. There is some evidence that certain diesel exhaust components are elevated in garages. This has been shown in several studies published now in the literature but the data is scanty. It needs more work and before anything was undertaken or as part of the undertaking of the study of these types of groups, I think that it would be essential to have some exposure measurements.

The other major group that I think is appropriate would be drivers of certain diesel vehicles, mainly truck drivers. These people have been shown, to a limited extent, to be exposed to diesel emissions in the cab at greater than ambient levels, and this is again very preliminary evidence with only a few measurements of carbon monoxide and nitrogen oxide and hydrocarbons taken in the truck cabs. The in-cab concentrations were compared with measurements taken outside of the cab either in the downwind clean environment or taken while the truck was on the road moving from a monitor that was attached outside of the truck, presumably away from the stream of the exhaust.

Again, there needs to be more work done but I think that there is evidence that there certainly could be exposure to exhaust products in the cab.

(Transparency) This lists some of the things that we are attempting to do. First of all, we are compiling the existing data and this is fairly well done but we're still searching for some investigations.

Secondly, we are trying to look at studies that are ongoing right now and there are a number of these in the United States, for the most part being conducted by the NIOSH group at the Morgantown facility. Of these, approximately four studies that I know of are underway from the epidemiology group, and three of these are examining mining populations while the fourth one is concerned with the mechanics of railroad engines. The reports of these investigators will be complete, as I understand it, within a short period of time, several months. The exception is the important investigation of railroad mechanics' mortality which is just underway and will not be completed for a year or more.

Those are the studies that I know of that are underway currently in the United States. Overseas there is one very large study that many people have been talking about and even though the final figures are not out, I thought I'd better review it since so much is being made of the London Transport Bus Workers Lung Cancer Mortality Study. In the next two slides I will briefly review that investigation.

(Transparency) This is a very interesting investigation because it's been taking place over a longitudinal period of 25 years.

It is a very large incidence study of respiratory cancer in the workers in the London Transport System. The London bus system was dieselized in the early fifties and at that time the British Medical Research Council entered into the picture. They have collected all of the cases of lung cancer that have arisen in the active worker population during the last 25 years and what they are looking at is rates of lung cancer in the several different groups within the transport workers.

They had a large number of cases--660. I consider that to be quite large and over those years they have adjusted these agewise to the greater London population and they've compared the rates to the greater London population lung cancer and mortality rates. The four groups that they are looking at are the mechanics, the drivers and conductors, the white collar workers and also the electric train workers who work underground.

The results (I do not have the numbers) show that all of the lung cancer rates in these groups are below the general population rate in Greater London and the analysis between the groups shows no statistically significant differences. However, there are slightly higher rates in the mechanics and in the electric underground workers, but again they are below the general population rate and they are not different statistically from the other two groups.

(Transparency) This transparency goes over a couple of other limitations of this study. I don't believe it is going to provide all the answers by any means. The obvious deficiency when looking at respiratory cancer is the fact that there is no smoking data and there appears to be no hope of getting any kind of smoking data. Secondly, it looked only at the age group of 45 to 64; when workers retired they were lost to the study. So, if there were a large amount of respiratory cancer that showed up very late, either because of an extremely long latency period or because of some differential effect in an older population as compared to a younger population, then it could be missed in this study. The dropouts from the population group were not followed. In other words, it looked only each year at those people who were actively working there.

The exposure data is very meager. There was one measurement taken in 1956 in the garages of smoke and of certain other polycyclic organic compounds and there have been no further measurements. They are going to repeat them this year again for the second time. And, the last point is the fact that there is some preemployment screening and the possiblity exists that susceptibles could be screened out becuase of their medical condition.

That's my understanding of this large investigation and we should have the figures within 1 to 2 months. I understand it is just in the final preparation now.

(Transparency) We plan on conducting several epidemiologic studies during the next year. There will be probably one and possibly two studies that are done by way of contract which will look at occupational groups and most likely they will be one of the groups that I mentioned. However, it will depend on the proposals that are generated. Hopefully there will be both a morbidity and a mortality component to these studies. In addition, we hope to replicate these or to perform similar types of investigations ourselves in-house. The mortality study, I think, to be able to get anything that would be able to have results within a year, would have to be very quick and dirty and use probably a case control design which would look possibly only at respiratory cancer. The ideal mortality study, which should get underway also, would be a historical cohort study. At the present time I cannot say whether data exists to do this on any of these population groups because it involves the necessity of very good records. There has been one historical cohort study that was published recently that took data from the Central States Pension Fund of the Teamsters Union and though it was quite limited in its methodology, it did point out various rates of diseases. It was not able to separate out occupational groups within the union.

Lastly, I am attempting to encourage other people, particularly researchers who have already looked at some population groups that might be dieselly exposed, to go back and look again to see if there is any potential to find out if the group can be studied from the diesel angle. This came up in a railroad retirees' mortality study and also it is a possibility in several other groups.

I am also still trying to identify populations that can be studied. There are several that are very up in the air right now, such as a Navy group and I just don't know if enough data exist there but we are trying to look into the possibility of workers on ships who might be exposed in engine rooms.

I'd certainly appreciate any helpful hints or suggestions on how to identify diesel-exposed groups.

DR. FLAMM: Thank you very much, Dr. Miday.

It occurred to me during your talk that something that Dr. Albert had said earlier today, concerning dosimetry, that one really didn't know, you know, what chemical constituent to look for but it seems to me and maybe we can talk more about that tomorrow, that it is possible through animal studies to determine whether there in fact exists a marker chemical within the mixture of substances that constitute the diesel hydrocarbons and that if one did have such a marker, one of the things you might be able to do would be to use it to see whether these population groups in fact do show plasma levels or blood levels that indicate clearly a higher exposure and this might be a better shot at it than trying to do it with the ambient environment of micro-environment in which they find themselves.

It also occurs to me, and this may just reveal my own naivete, that people sitting on the Long Island Freeway diesel materials might have considerably more diesel exposure than someone who isn't involved in that kind of traffic and verification that a difference exists could perhaps be done on that sampling of individuals and determining blood levels for, say, the marker hydrocarbon.

I don't know but it does--I think unquestionably there are large populations out there which could be defined possibly if we had the biochemical technique that would help to verify that they do exist and help to define the existence and also the levels of exposure.

It would also give all of the animal studies more meaning as well if, in fact, we could equate all those animal studies done generally at much higher levels of exposure to the human situation and it would tell us, not only about your high risk groups but what we might be able to expect from the population at large.

Having said that, let me see if the panel--yes, Ray?

DR. SELTSER: I suspect we'll get into this tomorrow. I'm constrained and I feel forced to make a comment. I think that some of the hopes that you just laid out for epidemiologic studios are perhaps something to think about for the future, but I think are quite unrealistic in relationship to the kinds of endpoints we've been talking about today and particularly in terms of the whole concept of applying epidemiologic data that might be available to the concept of risk assessment using cancer as an endpoint.

The people who have been, over the last 5 or 10 years, exposed to the Long Island Freeway, are going to be getting their cancers 5 and 10 years from now. The whole concept of identifying a population-at-risk means having enough years of exposure on the one hand, and use of followup for the development of health effects when you're using cancer as the endpoint.

I feel it is unreasonable to expect that you're going to be able to ferret out the variable of diesel exhaust from the other variables which have to be considered in every respiratory cancer study. Smoking has got to be considered the confounding variable of tremendous importance.

I see no hope really, for example, for any of the studies dealing with miners, to allow us to make any sort of inferences regarding the effect of diesel in metal miners or any other type of miners, considering the known carcinogenic hazards of miners for lung cancer. The smoking issue, the exposure to radon and other particles, et cetera, all of this, it seems to me, you're talking about diesel exposure which is perhaps the head of the pin on the major problem.

So, I feel somewhat pessimistic at this point. I don't think the London data is at all, frankly, relevant to this. Regardless of what they find in a reanalysis of this, I think the points that you've brought out in terms of being a very biased type of sample, only including the individuals who were in the population, not including the retirees, it invokes a kind of bias which, I think, could make it irrelevant in terms of trying to relate this to diesel emissions. I don't think that there will be, by the end of this year, any very useful epidemiologic data that is going to be meaningful put into this equation.

Now, I do have one comment regarding something that came up this morning and it does seem to me that you can use the epidemiologic method to perhaps try to get at something which relates to ambient air in these high risk environments.

From what I heard this morning, it seems to me if you're going to be doing studies, particularly on ambient air, looking for mutagenicity, et cetera, one might get two kinds of spin-offs with this.

One might be able to focus down on "high risk" air to be studied by some of these techniques into which we're pouring all of this money.

Secondly, the results of these studies might, in turn, lead us to identify those populations which are most amenable to study. I think that when you talk about the bus drivers, for example, I'm not sure just--maybe a bus driver in his cab is at more risk and maybe he's at less risk.

Perhaps a driver of a bus, since he sits at the front of the bus, may be at less of a risk than conductors who stand at the back of the bus closer to the exhaust fumes. But, I really do think that the possibility of doing something more with the ambient air exposures than has been suggested thus far might be a lead in terms of using the epidemiologic method combined with the laboratory approach to do something that might be done within 9 months to a year.

DR. MIDAY: Thank you for your comments. I am not quite as pessimistic as you are about the possibility of using epidemiologic data. I think that all studies are going to have limitations but I think that even something like the London study is quite important and has quite useful information for us and if we understand what those limitations are and we can take account of them, when we're looking at the data.

One of the points though is that if you can take into account confounding variables when you design the study, you can sometimes look at populations who have other exposures and still get at the variable that you're most interested in. Such as, if in the mining situation, there were mines being compared that were very similar except that in one mine diesel engines were used and in the other mine they were not used, then I think that the situation might be useful to look at health parameters and you could have a control over the other crucial variables.

DR. SELTSER: I certainly don't mean to say that I don't think epidemiologic studies are important in this area. I'm talking about the possibility of developing useful data for input into the short-term risk assessment procedure since you mentioned that there are really no new epidemiologic studies underway at the present time. On the basis of what you described and what I've read, I don't see on the horizon any data on epidemiology that are now on the road that are going to be very useful within the next 8 to 10 months. I certainly am a proponent of epidemiologic studies.

DR. FLAMM: Just as a point of clarification. I understand that we are concerned with more than just the January 1980 date, that there also is a concern 3 years hence in 1982. I think we probably should not limit our thinking just to what can be done in 1980 but also the 3-year period. But, I think we ought to be looking down the road also 10 or 20 years as well. It's those kinds of prospective studies where possibly, even though there are all these confounding variables, that one could conceivably establish some prospective studies if there are clearly areas where there is going to be a two-fold or threefold increase in diesel emissions. Very careful studies, where all the necessary factoring in is done, perhaps could provide some idea as to what health's burden, if any, insofar as lung cancer is concerned, or other cancer is being imposed.

But that would mean that the damage had already been done, for one thing, and it would also mean 2 or 3 decades before we could begin to have the information but that might be better than simply at some point in near time in the near future, saying that, well, we decree it to be okay and we're just going to go ahead willy-nilly and we're not going to attempt to make an assessment through prospective studies, if there are some that can be done.

It is conceivable that the capabilities that NCI has with its SEER program which is a population-based study, might be useful in providing some data which, while it may not be explicit to diesel fuel emissions, will give you a pretty good number in terms of saying that it's no worse than or it can't be any worse than, because of the data. I don't know, but I don't think we should restrict ourselves here just to a timeframe, given the importance of the question and given the need to have the best answer possible over a reasonable period of time.

Ray, did you want to comment further about the epidemiologic studies?

DR. SELTSER: I'll do it in the discussion tomorrow.

DR. FLAMM: Dr. Goldman?

DR. GOLDMAN: I though maybe we could end on a more popular note. There are two questions that I wonder if someone could answer, in the audience or in the panel, that relate to the quantitation of things that we've been hearing today. I've heard a lot about what certain milligrams of extract will do. I wonder if someone could tell me how many grams or milligrams of the fuel exhaust are necessary to get one milligram of extract. Secondly, what weight of fuel is necessary to get one milligram of extract?

DR. CORTESI: Ten grams of whatever collected on the filter produces about one gram of extract. The other figure I don't know. Does anybody?

DR. GOLDMAN: The second question is per unit mass of fuel, how many—per unit of material collected on the filter, how many units of mass of fuel have to be burned?

DR. SOMERS: About 1 gallon of fuel. We could say 10 grams of particulate.

DR. GOLDMAN: That gives 1 gram of extract?

DR. SOMERS: Yes, right. But it's a little on the low side, very roughly.

DR. GOLDMAN: Thank you.

DR. CORTESI: We deal only with factors of 10 around here.

DR. FLAMM: That's good to know. I ask that we have available tommorrow some sort of pert chart or flow diagram as to all the things that are being proposed so we can look at it in its totality and so that we can also get some feel for just what level of effort is involved in each one of those things.

So, hopefully, they will be able to, between now and tomorrow, put some of that together for us. If not, we will have to sit down and create our own laundry list of the various things that have been proposed. Are there any further questions among panel members?

(Whereupon, at 5:25, p.m., the meeting was adjourned.)

OPEN COMMENT AND QUESTION SESSION
Wednesday, December 13, 1978

DR. CORTESI: Good morning, ladies and gentlemen. Let's get started. The first thing this morning is public comments, vox populi vox Dei, and we believe it, so could we please have the voice of God. I expect these comments to be scientific and technical in nature, and I am going to ask Dr. Flamm to be the host and chairman for this as well as his panel here. Dr. Flamm, if you will, please.

DR. FLAMM: Good morning, ladies and gentlemen. I trust you all slept well and that you are prepared for us this morning, and we are prepared for you. Yesterday, when I asked whether any of you would have comments or questions from the floor, a number of you indicated that you would, and that is what this morning's session is for. So, who would like to go first?

Would you please go to the microphone and tell us who you are and your affiliation?

A VOICE: I am Robert Freedman, U.S. Bureau of Mines, Pittsburgh, Pennsylvania, at the Brewston Facility, and we are doing work at that facility doing control work on diesel engines using scrubbers and filters, but we are interested in the physiological effects, and would like to interface in any way that we can.

I would like to make a series of comments first on--well, they are just one sentence each. They may appear seemingly unrelated, but they lead up to a question of either William Pepelko, or Kirby Campbell.

First--well, we know that particle size is not strongly influenced by engine mode, that is, load and speed, and we have done some work at the Bureau--scale-up of a filter material used at the University of Waterloo. They use a series of materials, and we have picked one that would last a long time; because this is used for mining and underground you can use bulkier equipment than you can use on automobiles, and we chose a ridiculously large size to start with, and then scaled down. I won't go into the details, but we were able to remove 65 from the start and quickly going up to about 90 percent of the particulate, and we know that the mass median diameter of diesel particles is something like 0.1 or 0.2 micrometers.

In these tests, and also in our Instrument Development Bureau, we can measure on-line particle size like electrostatic charge instruments, and we find that there are shifts in particle size with this type of filtration.

Another way that particle size can change, and change very rapidly, even in the time that the particles leave the engine and go through a fairly long heated duct work to where we measure, and that is all we are interested in, is measuring before and after, mostly mass loading, but also particle size, and even in that period we haven't measured it yet but we intend to. It is known that even in the matter of a few seconds, particle size changes, and in a mine it may grow to several micrometers in length. There is some very scanty data on that. And, of course, in a workplace this would be true also.

Also, absorption of gaseous and liquid contaminants is not necessarily completely a bulk phenomenon, and in materials such as alumina and silica, it only goes down a short way or may be even unimolecular. With activated carbons it goes down quite a few layers, but not uniformly through the particle. Therefore there is a rather complex relationship also between the size and the area and the amount of absorption. So, it is not just easily calculable, a cubed function between size and particle mass.

All right, taking all those facts into consideration and then considering these chambers, in which you duct exhaust gas, are you not running into some problems in knowing where you stand when what you are measuring you are absorbing into solution, and so on? You are really checking and you may get more material out than is actually in there from the carbons, and are you not worried about the change in particle size? Do you know where you are at when you take chambers? We worried about that in the work done for us at Bartlesville Energy Center.

Another thing that John Johnson, who is here from Michigan Tech and who has written some papers on this change in particle size, noted is the effect of dilution, and the fact that in dilution tunnels you don't get anywhere near the same thing that you get in immediate dilution into the atmosphere, and the actual mass that you collect on filters can vary greatly with the amount of dilution. I know this is a question that is very difficult to answer, but I just throw it out to show the complexity of surface and mass properties.

DR. FLAMM: Go ahead, Dr. Campbell. Dr. Campbell, did you want to say who you are for the record?

DR. CAMPBELL: I am Kirby Campbell, Health Effects Research Laboratory of EPA, Cincinnati.

I am not sure if I understand completely because the answer seems easier than he indicated: We dilute our exhaust immediately after it exits the tailpipe into a dilution tube, and then it is distributed to a large-volume mixing chamber and then to all the exposure chambers. We don't bother with measuring particle size and particle characteristics in the hot part of this distribution train. We do our measurements of our gases and particle mass, and particle size--whatever we do in that area, so far--at the level of the animals in the chambers.

As far as loss of particulate material, yes, we have experienced some loss between, say, the mixing chamber and the animal exposure chambers, but that does not appear to be a serious loss. It is nothing like a large fraction. I don't remember what the figures are now. We can probably find out from our data, including more recent data, but it is not a serious loss.

DR. FREEDMAN: May I respond?

DR. FLAMM: Yes, sir.

DR. FREEDMAN: I wasn't referring merely to the loss in particles. And, also, it isn't only a change while in the heated state because we know in a mine, which is really a very large dilution chamber, that after you get the diesel out in a very high rate of speed, of the speed of the air stream due to ventilation, there is still aggregation and agglomeration over a period of time, and what I was trying to highlight is the change in chemical and physical properties that takes place. So, really, you don't have the same thing, or anything near the same thing, in a small enclosure you are talking about, as you would out in the atmosphere, or if you would in a dilution tunnel. That was really my main point.

DR. CAMPBELL: I don't think I can comment much on that. We use the chambers for obvious reasons to expose animals, and how it relates to the outside world is in an area beyond what I can cope with. I don't know, maybe Ron Bradow may have a word to say on that. But we have to do something.

A VOICE: Kirby's problem is pretty severe.

DR. FLAMM: Would you state your name, please?

A VOICE: It is Ron Bradow from EPA in North Carolina. The problem of the animal exposure is pretty severe. Once a particle loading in the exposed atmosphere is chosen, and you have a given emission from a passenger car diesel engine, then the dilution ratio is defined.

If you choose a particle mass loading of 6 milligrams per cubic meter in order to get some response--this apparently being the situation, we can't get a response from low particle loading--then the dilution ratio is whatever it is.

If there are issues arising from changes in particle composition or size as a function dilution ratio, then it is just uncontrollable in that experiment. Isn't that right, Kirby?

DR. CAMPBELL: Yes.

DR. BRADOW: That's what it is. Unfortunately, we don't have any way to control the particle mass emissions of automobiles to make them go higher or lower and still represent what happens when automobile engines run. So, the dilution ratio is chosen because of the particle mass loading desired for the experiment. This area, I think, still has some controversy whether or not the organic fraction associated with the particles is realistically defined by the sampling procedures that have been used in the variety of EPA experiments, both in my laboratory, in the Ann Arbor laboratories, and in Cincinnati.

The most recent experiments that have been done in my laboratory have measured the fraction of the organic material as a function--the fraction of the organic material of the total particles. The extract of organics is a function of the total particle mass at dilution ratios from about 5 to 1 out to 275 to 1.

Now, the high dilution ratios are hard to measure. The way this was done was to introduce an inert gas, N_2O, into the diesel engine exhaust, and to measure the N_2O concentration of the raw exhaust and of the diluted exhaust by electron capture gas chromatography. Our experience has been at 5 to 1, 10 to 1, 20 to 1, 100 to 1, and 275 to 1 dilution ratio. There the organic material associated with the particles is constant in fraction, constant in molecular weight distribution, and constant in mutagenicity.

At higher dilution ratios it might be characteristic of the ambient air, and perhaps there might be some other difficulties, but at least with the experiments we have done so far I think most likely the dilution ratio table does not introduce large difficulties with the trapping of these gases.

DR. FLAMM: Would you please indicate who you are?

A VOICE: Trent Lewis, with NIOSH, Cincinnati. Dr. Bradow, when you were talking about the removal of your particulate, I wasn't clear whether you were talking about catalytic scrubbers or water scrubbers. In the case of water scrubbers, I think you are going to change the characteristics of the particulate markedly, because you are going to humidify that surface.

Your point raised in terms of growing in size with time, and what have you, is not that atypical from the real-world situation, whether it be in the mine, or whether it be in environment. Those particles are very readily airborne, and remain airborne for a long time. So, even though we are ventilating the mine, there are some particles that are aging and, so, a miner will get exposed to freshly generated diesel exhaust particulate and, likewise, he will be exposed to aged particulate, and it is exceedingly difficult, and in all practicality, impossible to simulate to the nth degree the human exposure in an animal exposure experiment. We do as well as we can, but we cannot do it 100 percent, and I must emphasize that the particles that go through a water scrubber are markedly different in their toxicity and the reactivity adsorption properties of their surface, et cetera, than ones that would be emitted either from a catalytic system, or directly from the exhaust pipe into the atmosphere.

In our study at the Center Hill Facility of EPA, we will be looking at the water-scrubbed particles from the Caterpillar engine to assess the health hazards while they will be, in all likelihood, looking at the exhaust particulate, you know, nonscrubbed or catalytically converted.

A VOICE: I am Nicola Loprieno from Italy, University of Pisa. I am interested in mutation studies.

My question is mainly related to the type of approach which has been decided to be used for evaluation of the fractions which have a mutagenic potential, namely, the metabolic activation systems to be employed in the mutagenicity short-term tests.

To my knowledge, it has been demonstrated that the rat liver homogenate and the mouse liver homogenate (both S-9000) differ for the relative proportion of metabolic activating enzymes (the Mixed Function Oxidation) and the detoxification enzymes (Epoxide hydrase) and that the two types of metabolic activation systems do produce in benzo-a-pyrene, for instance, a different spectrum of metabolites with mutagenic activity, and only some of them are related with a carcinogenic activity. Have you considered the fact that by using only one type of metabolic activation system in the Ames test you could have a nonrealistic picture of the actual mutagenic components of diesel exhaust fraction?

Moreover, as a second question, you have planned to use in your project different short-term mutagenicity assays with the goal to identify the potential factors of risk that should be those which will be shown to have produced as many as possible genetic end points.

In your program, however, you are studying the induction of different genetic end points, by using a completely different metabolic system, as, for instance: UDS: S-900, from untreated male Swiss-Webster mice liver; mouse lymphoma mutational assay: S-9000 from aroclor-induced rat liver (strain unnamed); SCE: S-9000 from untreated Swiss-Webster mice liver; BALB 3T3 (Oubain resistance): S-9000 from aroclor-induced Fisher 344 lake rats; and salmonella test: S-9 mixed from aroclor-induced Charles River - CD1 rat liver.

They are five different tests in different biologically organized systems combined with five different metabolic systems.

In such cases the probability to find a positive correlation with different genetic systems must be highly reduced, to my opinion.

So, by using this approach, I think it would be very difficult to find a correlation, or if you found it, this could not be based on a rational basis because each one of these genetic systems may allow you to identify a different active fraction, and quite certainly will show a different mutagenic potential.

DR. FLAMM: Joellen, did you want to comment?

A VOICE: Yes.

DR. FLAMM: Would you first state your name and your affiliation?

A VOICE: Joellen Huisingh, Health Effects Laboratory, Research Triangle Park.

I think you have made a good point that in these comparative matrix studies we should be using a standard activation system. The arochlor-induced rat liver S-9 microsomal activation system has been the most widely used activation system with microbial mutagenesis bioassays. It provides a wide spectrum of metabolic activation capabilities.

To the extent that we can, in the diesel research program, we are attempting to use standardized protocols for test systems with as large a data base as possible.

I think your point is very well taken and we certainly will standardize the activation systems across the test matrix where possible.

The other point I would like to make is that our experience to date is that most of the activity we see is direct acting and does not require any activation. The addition of metabolic activation does affect the toxicity in both mammalian and microbial assays.

DR. FLAMM: Yes?

A VOICE: My name is Bob Biles and I am with EXXON. I would like to direct this question to the panel members, but specifically I believe that Dr. Campbell talked in reference to it yesterday.

We heard of a host of tests that are either being performed, or planned to be performed, specifically, some of the in vivo tests in inhalation, and possibly some of the in vitro.

My question is: Don't you think that some of these tests seem to be fairly exotic as far as mutagenicity goes, speaking specifically about tests such as alkylation of hemoglobin?

Ehrenberg has been working on this in Sweden, and some of the results he has gotten with ethylene and ethylene oxide are still questionable, as well as some of the other researchers in the United States that have been working with alkylation hemoglobin, so my specific question is: Why are you concentrating on doing some of these "exotic" tests, and some of the other tests that you are planning to do, rather than putting your manpower and your time into the more basic toxicology tests that need to be done with diesels?

DR. FLAMM: Go ahead.

DR. ZEIGER: I am Dr. Zeiger from NIEHS. Are you addressing that question to the panel or to Dr. Campbell?

DR. BILES: Either one.

DR. ZEIGER: It is more appropriate for Dr. Campbell since the panel is evaluating this and not defining. The panel has not developed these protocols.

DR. FLAMM: The panel does not necessarily disagree with you--with what you are saying. (Laughter.)

DR. CAMPBELL: Nor necessarily agree with Campbell. (Laughter.)

Well, we don't consider this an exotic test, and whatever other few exotic ones we might have, as a concentration on exotic measures. Our emphasis is on the outright induction types of tests and basic toxicity tests, noncarcinogenic toxicity, but we felt that where there was an opportunity to use some of these other indicator systems, we will do so if we can do so practically and in a nondestructive way, and without much effort in manpower and resources.

I am not an expert on these exotic mutagenicity-oncogenicity test measures, and I think for better coverage of this particular kind of question I would prefer to refer to our man who is working in that area at the laboratory, and that is Dr. Pereira. Perhaps, we could communicate something directly, or through the panel, on the discussion of this matter, but it is not a heavy emphasis in our program.

DR. CORTESI: Cortesi, EPA. I think in this particular case it is not particularly a diesel problem. It sort of comes from an experiment we are doing on the diesel things.

EPA, for obvious reasons, generally has a great deal of interest in short-term tests that can help us evaluate carcinogenic and mutagenic potentials, and we have these pussycats in these boxes, and they have a fair amount of blood compared to salmonella and other things like that, and they are just a nice thing to try out these new techniques and short-term tests on. It is not particularly directed toward the diesel problem.

DR. ZEIGER: Zeiger, NIEHS. I would prefer to use validated versus nonvalidated tests rather than exotic versus--I don't know what the opposite of exotic would be--benign.

I think we have got to beware of doing tests because a test exists and we have animals around. I think the tests have to be designed to answer the question. I think the last thing EPA needs is answers for which they cannot formulate the questions.

A VOICE: William Pepelko from EPA. The only comments I would like to make on this is that this test is presently being developed not specifically for the diesel program, so it is a developmental type of research, and the cats are not being exposed for this purpose, and if this type of measurement is developed and works out as a screening test, it will be used on the diesel.

As I say, we are not putting a lot of resources into this at this time.

DR. ZEIGER: Zeiger. Can I ask just a quick change in protocol that you change your alkylation measurements from the hemoglobin to the DNA regardless of the animal species?

DR. PEPELKO: I would have to refer this back to our expert, Dr. Pereira, and have him communicate with you on this.

DR. EPLER: I would somewhat disagree with Dr. Zeiger's view that he has put forth here. I think this is an excellent opportunity perhaps for the Agency, and perhaps for science in general, to look at what could be a technique that might actually give us some information in a timeframe that is sensible and in a financial frame that is sensible, that might really reflect on this assessment in man.

It is one of the few opportunities that we have to gather information in a whole animal, and to really see whether the results that we are getting in short-term systems in microbial, and fungal, and other so-called "submammalian" systems really have any appropriate analogue in whole-animal testing.

So, my view is, if that the research time is available, the Agency ought to go ahead and follow up these leads.

Secondly, you know, we have already been shown that the majority of identified components of this diesel particulate matter are polycyclic aromatic amines, or polycyclic aromatic hydrocarbons, or oxy compounds. On the work that I am aware is being done on hemoglobin activation, even hemoglobin alkylation, there we are talking about direct alkylating small molecules. I have no idea if any work has been done to show that any of these polycyclic compounds will alkylate hemoglobin.

This is what I mean by getting a lot of data, possibly negative data, that we will really not be able to interpret, or that EPA will not be able to interpret. We know these compounds will alkylate DNA. We do not know that they will alkylate hemoglobin.

DR. EPLER: I guess what I am saying is that I would hate to discourage anything that might approximate basic research in a mission-oriented project.

DR. FLAMM: Dr. Seltser?

DR. SELTSER: This raises what I think is an extremely important point.

Yesterday, in talking about the charge to this group, and what was to be done, I would like to paraphrase what Dr. Cortesi said to us when he said that the objective was to present in a public forum what the EPA health research approach is regarding the risk of introducing into the public domain a fleet of light-duty diesel vehicles. He indicated that we were to evaluate this health effects research program, and ask questions concerning whether the direction is appropriate and the methods adequate in relationship to this particular question dealing with risk assessment.

It seems to me that what we are getting involved with is something that is of great concern to me. We are to try to evaluate, under the context of one particular question, the adequacy or the validity of some very important basic research, or long-term objectives which, in fact, ought not be deterred from proceeding.

But, in terms of the specific question that we are being asked, it does seem to me that some of these basic problems, the allocations of resources, to what extent does some of this very interesting, and intriguing, and important research regarding the carcinogenicity of fractions relate specifically to this problem of the potential health effects of injecting into the public domain a fleet of light-duty diesel vehicles. How unreasonable is it to expect that the public is going to be subjected--to what extent is there going to be an unreasonable hazard imposed by this kind of an activity?

It seems to me a number of things have come up which have very little bearing on that particular question, and I would hope that we could at least focus and decide what we are going to be looking for, because I would also hate to see good basic research go down the drain because somebody says that this isn't relevant. Relevant to what?

DR. FLAMM: Well, this is exactly what our panel is going to have to discuss later as we deliberate toward the creation of a panel report addressing the charge that you alluded to.

Yes, please? Would you give your name and affiliation?

A VOICE: Charles Church, I am with the Army. We are a large diesel fleet operator, and also we have to buy United States equipment.

I was at the NHTSA meeting for the last few days where they are excluding the diesel from all considerations except overseas (Fiat).

My question is: Are we looking at it from an engineering point of view also?

One of the questions that was raised by Brian Ketchum, who was at the NHTSA meeting was, "Are we looking at a purely aliphatic-type fuel, or maybe even a narrow-based aliphatic-type fuel for diesels?" Are we looking at processes that take place in a combustion chamber that can create this kind of particulate; are there other means of regulation other than purely the regulation of the exhaust effluent?

Another question which I think needs to be raised is that the rule-making that is proposed deals with large particulates. The questions that people have been raising at this meeting have been concerned with very small particulates that go deep into the lungs.

The large particulates have classically been controlled in the diesel by virtue of the Bosch and the Hartridge Smoke Tests. Current rule-making is aimed at the large particulates which have a large amount of mass fraction in the effluent.

The current rule-making is aimed purely at the grams per mile, and nothing to do with engine size, where, historically, the heavy-duty diesel engine has been regulated for other emissions on the basis of grams per brake horsepower hour, or, in the case of the Hartridge of Bosch Test, simply the opacity of the smoke.

Are there any people looking at this problem from a mechanical engineering point of view, to see if there are control measures such as control of the fuel? Do the necessary technologies exist?

The specter that I see rising, of course, is that you think you are looking only at light-duty diesels. The issue that was raised in the Washington Post very recently is: "If light-duty diesels are bad, are also heavy-duty diesels bad? Are diesel farm tractors, ships, trains also bad? Are gas turbines and furnaces also bad?"

The issue that was raised in a recent Fortune magazine was, "Are we even looking at the right thing, or should we be looking at the transition metal sulfates which appear to have a catalytic effect in mammalian tissue?"

A question raised by Dow very recently was on the effect of trichlorinated dioxane, which apparently is considered to be carcinogenic, and is apparently an effluent of other kinds of cars.

DR. FLAMM: Thank you.

Would anyone from EPA like to reply to that?

DR. CORTESI: I didn't really hear the question but we have Joe Somers here from the Program, who is going to respond.

A VOICE: Joe Somers from EPA in Ann Arbor. I will make one or two comments, and then perhaps Ron Bradow would like to make some comments on the fuel work that EPA is doing, looking at various diesel fuels, and the effect of these fuels, and the parameters in the fuel in particulate emissions.

One comment made was that the light-duty diesel standards that will be proposed shortly for the 1981 model year will not be looking at any of these mutagenic health effects that have been raised in the past day or so.

The reason for that very simply is that the standard for light-duty diesel is to be effective for the 1981 model year, and in that timeframe, obviously, we cannot get an answer to the questions that have been raised in the past day or so on the mutagenesis of the particulates, and what this means in terms of an unreasonable risk to public health. Therefore, the 1981 standard is related solely to particulate mass and tied into the ambient air quality standard for total suspended particulates.

You have also mentioned the fact that the 1981 standard would be for light-duty diesel particulates. I would like to add that EPA is looking at the heavy-duty diesel particulate question, and currently plans to have a particulate standard for the 1983 model year.

Ron, is there anything you would like to add on the fuel work that is being done?

DR. BRADOW: Ron Bradow, EPA, North Carolina. Dr. Church has raised some very general questions. I think probably some of the things he said are relative to the evaluation of risk from various sources of carbonaceous particles discharged in the air, and are important questions.

It is true that light-duty diesel passenger cars will discharge into the air some particles, and that there are many other sources of carbonaceous materials in the air.

Dr. Roy Albert yesterday made the comment that probably any source of soot produced carcinogens, and that is probably right. He also made the comment that in risk assessment, one of the major uncertainties turns out to be exposure assessment, and at this point all I can say about that is that there is work going on now to model the various scenarios in which light-duty diesel passenger cars would be used, urban street canyons, expressways, parking lots, a variety of sorts of exposure scenarios, to allow us to calculate exposure in terms of concentration of particle mass. This work is going on right now at Southwest Research Institute.

There is some difficulty in constructing similar exposure scenarios for other sources of carbonaceous particles in the air.

Now, relative to the fuel program, the choice of fuels used to propel diesel passenger cars or, in fact, to generate combustion energy, is possibly beyond us. Petroleum is the source of material for operating diesel passenger cars. There are a number of processes that are possible to selectively choose certain components of petroleum for that purpose and reject others. It is possible that there are particular petroleum components that are the ultimate source of mutagens in the exhaust. However, we are hard put to design a fuel program that is sensible because we do not know what the mutagens are.

We do not know which compounds in the exhaust, particles or gases, con tribute to the mutagenic activity. Therefore, we cannot decide which compounds in the fuel might have given rise to those particular mutagen compounds in the exhaust. We are having one hell of a time designing a fuel program.

DR. CHURCH: There are some tests that Volkswagen did on their engine for DOT in which they ran seven different standard diesel fuels, and the emissions of smoke and hydrocarbons, which are measured differently, varied an order of magnitude or more over different operating ranges of the engine. This is with the same engine, under the same condition, with different fuels, all of them qualifying as diesel fuel.

As many of you probably don't know, the fuel that a diesel engine takes preferentially is an aliphatic fuel, a straight-chain carbon molecule.

The fuels which spark-ignition engines take preferentially include aromatics and cyclic compounds. Unleaded gasoline, with which many of you have become acquainted, is something like 40 percent aromatic.

If there is a theory of causality that does exist in a combustion chamber, the general stream of particulates, particularly an entrapping particulate, would tend to follow what's in the medium or in the chamber. The question I am raising directly to the group is: Are people looking at the mutagenic and carcinogenic properties of the fuels that would be purely aliphatic, purely straight-chain hydrocarbons?

DR. BRADOW: This is Ron Bradow, EPA, North Carolina. We have conducted experiments already with pure hydrocarbon fuels, particularly with normal tetradecane in a pilot experiment, a grant project, in my shop at Pennsylvania State University. We are conducting experiments in-house right now on the Caterpillar 3304 engine with a normal dodecane. Now, the pilot experiment so far with normal tetradecane indicates no mutagen release. We have negative results in the Ames Test with normal tetradecane. The dodecane experiments have a very limited amount of materials available from a single-cylinder engine, and we expect to have very substantially more material which runs with a larger engine, and larger sampling apparatus.

The single factor parameter, which seems to influence Ames mutagenicity to the greatest degree so far, has been fuel character. The particle mass emission rates do not seem, from a variety of engines, to be very much susceptible to changes in fuel character at all. However, the mutagen release is, and, consequently, the fuel is a very interesting matter.

The problem we face is that we cannot sensibly design a fuels experiment. We do not know which property of the fuel is related to the mutagen release, simply because we do not know what the mutagen compounds are. So, we are in the position of probing around in the dark, and we may select a variety of fuels having different properties on the basis of inspection tests, which have possibly some relationship to the premutagen precursor, probably not.

In a short time, short of identifying the compounds in the exhaust which are mutagens, and in tracing these to the field, it makes reasonable experiments with fuel properties just impossible.

DR. FLAMM: To what extent do you plan to try to identify the chemical structure of some of the mutagenic constituents?

DR. BRADOW: Of course, this has been the major effort of my research group in North Carolina for about a year and a half. However, for the past 3 months solid, our research program has been completely shut down in order to produce biological test material. The larger the demand for biological test material, the longer we will be shut down. We are not working on that right now.

DR. FLAMM: Dr. Seltser?

DR. SELTSER: I would like to be sure I understand what I just heard. What I think I just heard is somebody saying that in fact when one looks at the difference between gasoline and diesel engines, we may be talking about the difference in the fuel use and not a difference in the engines themselves.

DR. CHURCH: May I offer one more comment here?

DR. FLAMM: If you will go a little slower, please?

DR. CHURCH: All right. If what we are talking about is entrapped species on the particulate, that's one set of problems that you can control by the fuel use. If what you are talking about are species that are created in a combustion chamber, that's a different kind of a problem we should address.

That there ought to be some engineers in this work that know about the diesel engine itself and its characteristics.

There are some procedures we can do to control the gross particulate flow. This would be going to air heating, going to leaner stoichiometries and to higher injection pressures. There are other things we can do in the fuel line itself. If it turns out to make the light-duty diesel viable in the United States, you have to have a certain special fuel, then we know on whom to put the problem.

I would hate to see us lose the diesel engine for the wrong evidence. Thank you.

DR. FLAMM: Yes?

DR. FREEDMAN: Could I respond?

DR. FLAMM: Yes. Use the microphone, please.

DR. FREEDMAN: Bob Freedman, Bureau of Mines. We have been interested in trying different fuels, and we did try kerosene versus Number 2 diesel fuel with a Caterpillar engine.

We investigated this using the AD Little DOAS procedure, which is liquid chromatographic, proceeding on only two peaks, one presenting hydrocarbons, and the other oxygenates.

There wasn't any great change in the use of these fuels. Also, at Bartlesville, they investigated the formation of BaP, benzo-a-pyrene, and it did not change greatly.

Also, at several of these diesel conferences it has been said that a good part of the particulate that is collected can come from the lubricating oil, so we have to be a little careful in thinking that changing the fuel may have a great effect, or what are we talking about?

DR. FLAMM: Okay. As I recall it, the difference was approximately five-fold in mutagenic effect from the different fuels that have been tested thus far, which is a fairly significant factor, of course, but as Roy Albert kept emphasizing yesterday, first one runs a risk assessment which is within one or two orders of magnitude before you really begin to zero down on precisely what the public health burden might be. Obviously, that is going to take a long time to prove, so while clearly the fuels are important, we are not dealing with an importance that ranges over several orders of magnitude.

DR. CORTESI: I think one thing to keep in mind here, speaking for regulators and not scientists, a factor of 5 to a regulator is very big, and a factor of 5 to a scientist may not be very big, but when you get down to regulating, you just can't say that you are not going to pick up a factor of 5 because the data behind it is only good to a factor of 50. You are going to pick up that factor there, and that's the way the pressures work.

DR. FLAMM: Yes, please?

A VOICE: I am Dick Klimisch from GM Research, and I have two questions. One to Ron Bradow: You described some fascinating experiments yesterday about artifact formation on Chromabsorb 1 and 2, and you mentioned that this stuff occurs at the same place in a liquid chromatogram as does the active material.

The question I have is: Doesn't that suggest that the active material may also be an artifact that is produced on the filter in the normal procedure?

The other question I have got is to Dr. Seltser who said that he felt the London Transport Study was irrelevant because I think he said: "We are looking at the head of the pin," but perhaps that may suggest the size of this thing, that it isn't a large effect.

DR. FLAMM: Why don't you respond, Dr. Seltser?

DR. SELTSER: I'll be glad to respond to that. My comments yesterday, I think, should not be taken perhaps out of context. What I really was saying was directed at the prospect of developing new evidence that could be forthcoming between now and next December. I was limiting myself to the short-term goal.

I think, in fact, if you look at the London Transport data, with all of its inadequacies, the fact is that the London Transport data does not show the kind of evidence regarding the health effect of diesel that has been consistently demonstrated in other types of epidemiologic studies where you have a known carcinogen impacting upon the population, and where studies, even though they are poorly designed, generally turn out to show a difference even though it is not a statistically significant difference.

I know of no good human study of smoking which has not been positive, and it seems to me that the London study is of use in terms of being the only epidemiologic study which is specifically directed at the diesel problems, which has been a followup study.

One cannot prove that diesel engines are safe. I mean, if you indict something you say it is guilty until proven innocent. You cannot prove that diesel engines are safe by this kind of epidemiologic study.

There may be an effect, but the magnitude of the effect, it seems to me on the basis of the only human data we have available, is not of the order to give us the same concern as that obtained from epidemiologic studies of cigarette smoking, the ionizing radiation, and a number of carcinogenic substances, such as asbestos.

So, from this standpoint I really would like to be sure that I was not misunderstood in terms of saying that the London study is worthless. I was really addressing myself to what was, in the material we got, a proposal to reanalyze and to rework the British material, and my concern was that I really don't think that there is going to be anything more specific come out of that study over and beyond what we now have.

DR. FLAMM: Yes, please.

A VOICE: I am John Engel of Chrysler, and I would like to compliment EPA on including a variety of engines in the matrix to be studied in the early part of this program.

I think it represents a responsible approach and a recognition of the fact that there are all kinds of diesel engines, and that we should avoid referring to diesel engines as a generic class of machines, all of which act the same, all of which have the same properties in terms of introduction of particulate and organic matter into the atmosphere.

That same consideration applies, of course, to engines that are now on the drawing boards that will be part of the vehicle production in the future.

They also will vary, and may vary considerably relative to the kinds of properties that we see in diesel engines now. We do not currently have any unifying factors to consider the mutagenic and carcinogenic properties of those future engines, of course, as compared to those engines that are included in the matrix now, except for the possibilities that lie in Dr. Bradow's work.

I am very disturbed to hear there has been an interruption in that work. I just hope that the interruption will be temporary, and that work will be resumed as quickly as possible after the generation of particulate material for the biological tests has been carried out, because if we can find the chemical entities that are responsible for the mutagenic and carcinogenic activities, we may have something to refer to on analysis of future engines.

DR. FLAMM: Well, I think what you are saying is that we have apples, oranges, and grapefruits, and as we attempt to study these things, we really do need something fundamental to key on so that we can understand the interrelationship of the apples to the oranges to the grapefruits, and that the work which was pursuing exactly that has been delayed so that other activities could go forward. I don't think there is any disagreement but we are at that stage. Possibly, one of the utilities of the Ames mutagenicity work is that it will provide, ultimately, when we have additional answers, a quick way of assessing this kind of common threat or commonality, perhaps.

Yes, please?

A VOICE: I am Eugene Weaver of the Ford Motor Company, and I would like to make an observation based upon some work we have done, and express a concern.

The observation is that we have begun working with polynuclear aromatic compounds from diesel engines and PROCO engines, in connection with samples of particulate matter from these engines. The observation is that the nature of the filter paper, or the filter being used, usually a glass-fiber filter, has a considerable effect on the stability of the material collected on that filter, and we found that from 40 to 90 percent of a radioactive BaP compound, which is collected on the filter, disappears in a rather short period of time even when it is stored in a dark environment.

This observation leads then to the concern that the material on which all these medical studies are based is very ill-defined as far as what the specific compounds are that are causing the activity, and whether or not the material which you are really testing is the same material which comes out of the tailpipe of the vehicle.

I realize you have safeguards introduced which would ensure that the material doesn't change grossly during this series of experiments, but that isn't quite enough in my opinion.

DR. FLAMM: Yes, please?

DR. BRADOW: We have been in contact with Dr. Pearson at the Ford Motor Company in the last 6 weeks or so, and we have been aware of the experiments relative to the loss, particularly of BaP, on filtered medium and, in fact, have conducted experiments of our own which completely agree with Dr. Pearson's experiments, namely, that in any filtering medium we have used so far there is a very substantial loss in BaP.

However, I believe that the kind of phenomenon we are dealing with here is very similar to that experienced by Matt Norris and Rod Spin at Gulf Research in trying to measure BaP previously. BaP is a very difficult compound to handle in sampling. As a matter of fact, the issues right now relative to the sampling of mutagens are really unresolved.

One thing that has been sort of helpful to us at this point is the observation that at least in the extracts the mutagenic activity seems to be relatively stable by the time we get it into the X-ray form. Although there have been some storage losses in activity for rather casual storage conditions, and whatever the activity is, it is not lost very rapidly once--either on the filters or on extracts. The issue that I feel is more significant is just how much emphasis should we put on the hydrocarbons, anyhow? The fraction in which the mutagen is contained is not a hydrocarbon fraction. The fraction which contains BaP is not very active and, in fact, can't account for any significant amount of the overall mutant Ames activity, anyhow. As a matter of fact, a direct analysis for BaP in the extracts can account for less than 1 part in 1,000 of the mutagenic activity, so the hydrocarbons just may not be a significant source of mutagenicity.

DR. HUISINGH: Joellen Huisingh of Research Triangle Park. In the process of generating and collecting about 30 filters a day for weeks to obtain a large sample, Drs. Bradow and Zweidinger have selected five samples per day over separate days for bioassay. We have bioassayed each one of those samples separately to compare activity between filters collected within one day and between days. The samples will be stored and reassayed after storage. Frankly, so far, we see no significant differences within days or between days, between filters. The material seems to be more stable than some of our positive control compounds.

While I am aware of the problem that was discussed with BaP spiked on filters, it is not clear that once these organics are in the particle matrix, if they are stored on the filter in that matrix, that they are not stable.

At least, it certainly appears to be more stable than has been the reported situation for cigarette condensate, where a considerable amount of worthwhile research has been conducted in spite of these problems. While I feel we should continue to address the question of stability, we don't feel the evidence precludes continued biological research.

DR. FLAMM: Yes, sir?

A VOICE: B.C. Pal, from Oak Ridge National Laboratory.

I have several questions and comments essentially for Ron Bradow: We realize that the preparation of the sample is very, very important, and I wonder whether the diesel fuels that are obtained from different kinds of crudes, such as Russian crude, or Middle Eastern oil, or American oil, should be checked or not, and also whether a particular batch of Gulf Number 2 diesel fuel used for preparing the samples should be set aside for this purpose or not.

My other suggestion is that, since Ron indicated the difficulty in preparing samples from gasoline exhaust, we should try to mix the diesel exhaust with gasoline exhaust, and then you will have the particulates, and so, the gasoline exhaust will be deposited on this particulate and you will be able to process them. Because you know that almost 100 percent of the particulates are coming from diesel, you will be able to estimate how much exhaust you are getting from the diesel versus how much is coming from the gasoline.

This has probably some rationale because in the transition period we will be exposed to both gasoline and diesel exhaust.

The other point that I have to make is the particulates that are present, especially the inorganic material, and the organic that could be associated with the particulates after extraction with methylene chloride, are really important parameters to be looked at. They should not be ignored completely.

It is known that many cations, for instance, are picked up by physiological fluids such as serum, and in our experience we have seen that many metal ions are picked up by serum from asbestos particles, for instance. And, many hydrocarbons such as BaP, which is practically insoluble in water, are also picked up in serum, and we have found that in our experiments, and these data have been published, benzo-a-pyrene solubility in human serum is about 225 micrograms per ml. So, what I think is that this part of the exhaust should not be ignored completely. The metal ions and these organics that are associated with may play some cocarcinogenic or promoter roles.

The other point is that one should make sure that traces of methylene chloride are not left in the samples that you prepared and, also, the residue that is left after extraction with methylene chloride should be analyzed for the presence of carbon, hydrogen, oxygen, sulfur, and also the metals, and see if any hydrocarbon is left unextracted by methylene chloride, because it is known that some hydrocarbons are extremely insoluble in many solvents. For instance, pentacene is soluble with very great difficulty even in benzene.

DR. BRADOW: With regard to the inorganic compounds of diesel exhaust, first, diesel exhaust particles, the engineering program to assess the yield of sulfate nitrate ion, and other anions, and various types of metals have been going on for a number of years, and there are good emission factors for these substances available.

Generally speaking, there is not much cause for concern there. We have a number of papers we will be glad to supply you with on that subject. With regard to the nature of the solvents used in the extraction process, we have used historically back-to-back solvent extractions with at least two solvents.

In the initial work, methylene chloride was--really, a lot of the reason for choice of solvents goes back to the history of handling combustion deposits, lubricating all sludges or combustion chamber deposits. There have been some solvents that have been worked at over the last 50 years that are pretty good for handling most of the components of those kinds of effluents. The two that we have chosen were methylene chloride and acetyl nitrile. Acetyl nitrile is very good for dissolving these other substances of higher polarity and intractable polynuclear material.

We do get some additional organic material out with acetyl nitrile, and separation of this from the organic material by back extractions has yielded a substance which--an overall cut--is quite a lot less mutagenic than the methylene chloride extracts have been.

As far as traces of methylene chloride, one of the reasons for using that solvent rather than others is it is very easy to get rid of. High-grade, pesticide-grade methylene chloride with essentially no background, in terms of the stripped-down behavior of the residue, is available and has been used exclusively in our work, and we just haven't seen any background. In fact, background or blank samples have been run in most of the assays with no effects whatsoever.

DR. FLAMM: It has been brought to my attention that we have had open discussion now for approximately an hour, and during most of that period we have been dealing with characterization of the diesel emission rather than health effects, and while we do recognize that these two things are very closely interrelated, and you can't really do one without the other, the emphasis and prime focus of this activity is on health effects.

It has been pointed out to me that apart from the gentleman from EXXON, who expressed concern of what he described as some exotic tests, there has been very little in the way of criticism of proposed projects and programs. Absent such criticism, the EPA will assume that this audience, at least, is providing tacit approval, so that if you do have concerns with respect to the research projects, planned projects, then this certainly is the time and opportunity to express those concerns. It would be appreciated by the panel, and by EPA, if we could now begin to give that somewhat greater emphasis.

Does this relate to your last comments?

DR. PAL: No. I have another comment to make.

DR. FLAMM: Okay. Do you want to take the microphone?

DR. PAL: This B.C. Pal from Oak Ridge National Laboratory.

The epidemiological studies on asbestos exposure have focused attention to the high risk that the smoking population has for contracting lung cancer, and I wonder if in our diesel exhaust emission studies the cigarette smoke should be given more attention than has heretofore been paid for as a cocarcinogen, or promoter, and things like that.

DR. FLAMM: Does anyone on the panel wish to comment?

DR. WILEY: I believe, in my opinion anyway, about 90 percent of the program that we have been presented here is designed to answer a very immediate question by 1980; I believe, and I don't think that the cigarette smoking question should be taken into consideration of what is the immediate concern.

DR. FLAMM: We are going to break just for 5 minutes, which will give some of you an opportunity to get to the coffee machine. But, please, let's not go very far away because we want to come back in just 5 minutes.

(Thereupon, a short recess was taken and then the proceedings continued as follows:)

DR. FLAMM: Ladies and gentlemen, it is time to come back to order. If you will take your seats, please. (Pause.)

Again, I would like to ask that we try to give emphasis to the health effect aspects of the diesel emission problem.

Who would like to go next? Yes, sir?

A VOICE: I am Bob Wheeler with NIOSH in Morgantown, West Virginia. I am the project officer for the diesel coal mines study.

We have heard many people talk about the work we (NIOSH) have done in the mines, and I think it might be interesting to hear what we found. Dr. Wiley encouraged me to talk about what we've done, and what they've shown in some of their work at Battelle.

We have, up to this time, surveyed about 950 coal miners in six diesel coal mines in the United States; one in the east, five in the west. Of particular interest is a diesel coal mine in the State of Colorado where 28 percent of the coal miners are women, young women, who have an exposure of about 2 years. We completed our survey of that mine last week. Our preliminary results show very little acute changes in pulmonary function of the people who work in the mine.

We have completed medical studies in 22 metal and nonmetal mines. The nonmetal mines included salt, potash, and trona, while the metal mines include copper, iron, and gold. We have shown some pulmonary changes in the people who have been exposed to NO_2 concentrations in approximately 3.5 parts per million over an 8-hour shift.

Those are the only comments I would like to offer. If anybody has any questions about what we have done, I would be happy to answer them.

DR. FLAMM: Dr. Wiley, would you care to comment?

DR. WILEY: Wiley, Battelle.

I think these studies, these epidemiological studies, which were described, have to be taken into the context of studies that we have been conducting relevant to diesel--the use of diesel engines in coal mines, and relevant to the use of diesel engines in uranium mines.

We have conducted studies with diesel particulates as a part of these studies in the controlled experiments, and looking at these controls we have had particulate forms ranging from 6 milligrams per cubic meter to about 12, and these studies have been conducted with rodents, and we have used hamsters and rats.

In these studies we have not seen any tumors at all over the lifespan of both the rat and the hamster. The studies--you say, "How much did these animals get?" They were placed in chambers, and they were there for a period of 7 hours a day, and exposed for 5 days a week, so that the exposures are fairly high. There are some complicating factors, of course, because the NO_x is high, and all of the characterization problems that we have discussed, this is diluted to the level to get the 6 to 12 milligrams per cubic meter that we observed. However, we have not observed any tumors.

Now, I feel that these studies have to be rationalized in the experimental protocols which EPA is proposing at the present time.

In that connection, I was surprised yesterday to learn that a protocol to look at particulate deposition and turnover studies, which was included in the document which was sent to us, was not discussed at all as a part of the proposed protocol. I am speaking particularly of a piece of equipment which was designed to produce particles to simulate those of the diesel engine, and to attempt to add back to these fairly clean particles some of the fractions which have been shown to be mutagenic in the Ames and other in vitro studies.

I would like to have someone from EPA comment on the reason for the absence of discussion of these deposition and turnover studies since, obviously, the animal experiments which we have conducted say that the tumorogenicity of some of these particles is rather minimal.

A VOICE: Judy Graham from EPA. I would like to address your question, Dr. Wiley.

There was an original proposal to perform deposition and clearance studies. The reason it wasn't brought up was because there were a number of proposals, and they are ranked for priority to match the resources, and the priority ranking was not high enough to use some of the resources available.

Basically, just for the audience's knowledge here, I might very quickly mention what the study was: The National Bureau of Standards built a burner for us, and this burner will burn a pure fuel such as propane, and it produces a carbonaceous particle of similar physical properties to a diesel particle. So, with a system that will generate a pure carbonaceous particle, it then becomes possible to form any number of studies. We happened to think the most appropriate study to begin with would be a very quantitative deposition and clearance study.

Such a study could be performed on normal and impaired animals. For example, if an animal were rendered emphysemic, it might be possible to see if an emphysematous animal would have different deposition and clearance patterns, in which case one might assume that a human with emphysema would have different deposition and clearance patterns.

Maybe it would be indicative--it would help design an epidemiological study, that type of thing. It would also be possible to absorb organic compounds onto this particle, and also look for desorption in vivo. So, a number of studies could be performed.

We feel that the work would be relevant, and it would help make some of the quantitative aspects of inhalation exposure more relevant.

In other words, if we know that a certain concentration in a chamber results in a certain deposition, then we might know what kind of deposition resulted from the animal inhalation studies that are being performed in Cincinnati, and one might be able to include what might occur in the natural environment given the ambient concentration of what would be inhaled by the human.

So, these extrapolations are difficult, very difficult, but at least the extrapolations might be based on some experimental data instead of simply on theory, so it would be very difficult but I think we would be able to do better extrapolations than we could without the study.

DR. FLAMM: Yes, sir?

DR. ZEIGER: Zeiger, NIEHS.

I would like to thank Dr. Wiley for bringing up this point. It was a very obvious gap in the entire mass of paperwork put forth on this meeting.

I think this is what we are really asking, and not what methylene chloride extracts will do, but what lung extracts and stomach extracts will do, and for that we have to know what the deposition level is in ambient air, or in the chambers and lifetime studies, and we have to know what the elution rate is, and what the elution pattern from these deposited or ingested particles is.

I think this might be the key to tie together the inhalation studies with possibly the ingestion or in vitro studies.

DR. FLAMM: I was wondering whether you had any experience with this model, that is, I assume it is inhalation, and I was just wondering whether you have had any experience with cigarette smoke in this particular inhalation model.

DR. WILEY: We have had experience with cigarette smoke in the sense that we have been able to look at a particulate deposition from cigarette smoke through a smoking machine. I think that the machine that the EPA is talking about is quite different, but such deposition studies have proven to be useful in determining some important aspects of what the carcinogenic component is for cigarette smoke.

DR. FLAMM: I was wondering also whether you were able with that model to produce bronchogenic tumors with cigarette smoke?

DR. WILEY: I am not sure of the data from these experiments. These experiments are just being completed at the present time. So there are tumors but I don't know whether they are oncogenic, or otherwise, nor do I know what the incidence is now.

The deposition studies, of course are important no matter whether we get the tumors or not.

DR. FLAMM: Sure, absolutely.

DR. SELTSER: I just want to clarify what you said. Your studies did show tumors of some sort from cigarette smoke and no tumors from diesel exhaust; is that correct?

DR. WILEY: Yes, that is correct, no tumors. I was trying to recall whether--look, you know, you do see a large amount of hyperplasia and metaplasia. The lungs of these animals that have been exposed to diesel particulates are a holy mess. I mean, they are black and they are terrible, but most of the effects that we have seen in terms of the biological effects can be accounted for by the coal-only exposures. In other words, you see the same thing with coal: the interstitial fibrosis. We have not seen tumors.

DR. FLAMM: Yes, please?

A VOICE: Holdsworth, American Petroleum Institute.

I think we are finally getting around to the point that concerns me, and I speak not just for the American Petroleum Institute but primarily myself. That is sort of a disclaimer.

One of the things that has concerned me in diesel and diesel emissions is the biological approach that has been used so far, and that is mutagenesis and carcinogenesis, and in whatever has been done, largely extracts of these materials have been used.

Now, as a scientist wanting to take a realistic, pragmatic approach here, I would like to see somebody, and maybe this has been done, but I don't know because I didn't have the good fortune of being here yesterday, but I would like to see somebody take a look at whole diesel emissions. What really happens in mutagenesis, Ames Test?

I am a believer in the Ames Test and other mutagenesis tests. They have their shortcomings in improper hands, but they are good prescreening tools.

I would like to see somebody take the whole diesel emission and see what happens when one uses this. I would like to see it done in skin painting, and as Dr. Wiley has mentioned there, this is being done somewhat by inhalation. I think this is necessary.

I think to continue to extract these particles with various organic solvents, and then submit them to various type tests, is not a very real-world approach to things.

I really don't have any specific questions for anybody. This idea has been addressed earlier. I addressed this in a letter to Dr. Huisingh. API has made overtures to the Triangle Park Research Group, Matthew Bills, et cetera, that API is open and willing to talk to EPA and the Triangle Park group, in terms of research efforts, and I would like to go on record as saying we are still open. I think there are a lot of things that can be learned by interacting with API and with the automotive industry, both of whom have had a great deal of experience in these areas, and I'd just like to leave that with you. Thank you.

DR. FLAMM: Thank you.

Yes, Dr. Church? Dr. Church, could you speak a little more slowly? (Laughter.)

DR. CHURCH: I'll try.

Another question: I don't know if your protocol includes diesel extracts extracted from the engine as running over the federal driving cycle. The reason I am stressing that is the federal driving cycle primarily involves the engine running at a very high air-fuel ratio which may have a very different particulate type than you would expect for the engine running under full load.

For experimental convenience, particulates are obtained from diesels by running the engine under a heavy load, but on the federal driving cycle the engine is under a heavy load for a very small fraction of the time which is wholly in the acceleration.

When the engine is cruising steady-state at a light load, or when the engine is idling, it is running with air-fuel ratios of 150 to 200 to 1.

DR. HOLDSWORTH: No question asked! "Yes" agrees with the principal idea he pressed.

DR. FLAMM: For the record.

Yes, please?

DR. HUISINGH: I would like to comment back to Dr. Holdsworth from API. This is Joellen Huisingh with Research Triangle Park.

We very much appreciated your letter and the data that you sent us on bioassays you had performed on diesel fuel. I am sorry you weren't here to hear the presentation yesterday, and I would be glad to provide you with the information that I did present.

I think that we have been very concerned. We have done some bioassay work with carbon black and soot that we described yesterday, and I think that your concerns are real, and they are similar concerns that we have and we are trying to address them in our program.

Rather than to take the time to re-present that to you here, I would be glad to show you how we tried to incorporate your suggestions.

I would like to emphasize that the exposure time in all the short-term bioassays is very brief compared to the potential residence time for the particles in the lung. When the mutagenic or carcinogenic substances are in a matrix like the carbon matrix, the organics can't very easily leave the matrix, of course, they won't leave the matrix and the test will be negative.

What the relevance is to a particle that is in the deep lung with a very long residence time--you know, we are trying to approach mimicking that, but I don't want to fool you that through physiological extractions of any kind, or in vitro cellular extractions, that we can really mimic the in vivo long-residence time that the particles may have in the lung and I believe there is evidence these particles get into the lymph nodes.

What types of organics may leave those particles under those conditions? Our approach in the use of organic extracts is to try to separate the problem. When we are bioassaying the extracts we are saying, "What is the relative hazard of the organics?"

It is a separate question that, of course, has to be dealt with: Do those organics leave the particles and ever hit a target cell in humans? That must be addressed and is addressed in other parts of the program, and to the extent that we can, we hope that our program has put together a matrix out of using combined in vivo and in vitro tests that will help us to assess this in the end.

DR. FLAMM: Yes, sir? I'm sorry, the other gentleman behind you and then we will go to you immediately after.

DR. HOLDSWORTH: I guess that is my point, Joellen. If the polynuclears don't leave, or whatever the carcinogenic material or mutagenic material is, if they don't really leave the particle, do we really have a problem? You know, they may be adsorbed, they may be absorbed, they may be chemically bound. Who knows?

I would also like to point out, just for the audience, that we can't just equate each particle with another particle. Seemingly, every time these discussions come up we get back to good old Percival Potts, and most of us realize that soot from coal is carcinogenic if rubbed on the skin for any period of time.

That is not to say that we can necessarily equate soot with carbon black, with diesel emission, with other types of emissions.

One shouldn't necessarily think that one fuel is the same as another fuel. It is going to be quite different.

Coal is different from petroleum, petroleum is different from crude oil, and, folks, if you think we've got problems with coal and petroleum, wait until we get to shale oil.

API has had a lot of research experience. We spend millions of dollars every year doing things besides taking OSHA to court and lobbying in Congress. We spend about $4 million in environmental and occupational health-type research, so we do have a little experience in this area. Thank you.

DR. FLAMM: One of the things we want to hear about this morning is the research that is going on that does, in fact, relate to the health effects of diesel emissions.

So, if any of you who are engaged in such studies care to tell this panel about the studies, we would be more than happy to hear about it. I repeat myself because this is the time to express your concern regarding whether the planned studies will meet the stated objectives.

This (indicating) gentleman had a question.

A VOICE: Fred Benjamin, National Highway Traffic Safety Administration.

I am concerned just with the point that the chairman brought out now. Are these considerations really relevant to the decisions that have to be made? This panel has the task of evaluating a program and afterwards, come up with recommendations which serve as the basis for their decision. However, the concern of those studies is largely with diesel exhaust, and the decision has to be made on the basis of the atmosphere that is contaminated with diesel exhaust.

However, when it comes to exposure, apparently, this panel is not concerned. Is it not necessary that you make certain assumptions on what are really the exposure limits, and base your evaluation and recommendations on the basis of the actual or assumed exposure to diesel exhaust?

DR. FLAMM: I think the panel cannot ignore the issue and problems that surround exposure, but it is my understanding that there are other groups that are focusing on the characteristics of the emissions themselves, but I think we will have to, in our deliberations, consider very carefully the matter of dose from a given exposure, and this is something about which we apparently know very little, and it will hinder and hamper our ability to understand the interrelationships between the different things that have been proposed with different test systems.

Are there further questions?

DR. ENGEL: John Engel, Chrysler.

I think EPA is to be complimented for its zeal in attempting to get some results to us in a year to help us in our considerations relative to diesel-engine planning and design. I am afraid, however, that the results which are going to be available in a year in this program are not going to be particularly helpful because they are going to be mostly based on extracts from diesel exhausts rather than from experiments of the whole diesel exhaust itself, due to the time constraints and the latter studies.

It seems to me, what is lacking in the studies which would be very valuable in this next year, and perhaps achievable in the next year, would be the deposition and clearance studies which were mentioned a few moments ago, in combination with studies of the rate of extraction or elution of these organic materials from the surfaces of the carbonaceous materials produced by the diesel engine.

The comparison of those two rates will lead us to some potential conclusions relative to the availability, as Dr. Huisingh mentioned earlier, of organic materials to the tissues in the body, and until we have information like that, in the first year of this program, all we are going to have is the data on the extracts themselves and their effect on the body, which I think we already probably know, and tentatively some of the conclusions. Thank you.

DR. FLAMM: Go ahead.

DR. CORTESI: Actually, we are a little bit better off than that, I think.

We know that when human beings breathe cigarette smoke, and breathe coke-oven smoke, and breathe roofing tar smoke, they get lung cancer. Hence, when we perform these comparative experiments they are not perhaps watertight in terms of applying cause and effect, but they can, and I think in a very real sense of the word, give you some idea about how diesel particles compare in potency with these other particles which are, indeed, known to get into the lung and give people lung cancer, whatever their elution rate is, whatever their transit time is, and this and that.

So, we are better off than I think you implied when it comes to making a regulatory decision on the basis of data that we may have available here.

DR. FLAMM: Yes, Dr. Jones?

DR. JONES: Jones, Lovelace, ITRI.

I would like to comment on this whole question of deposition and retention of diesel emission particles. I, too, am concerned that these deposition-retention studies were afforded a low priority and thus not conducted. I feel that this is very unfortunate in that the particle size distribution, that I understand you are talking about, is probably the particle-size distribution that we know the least about relative to human deposition and retention. In the absence of this information, modeling efforts are virtually impossible because the fractional or the regional deposition could vary from 90 percent nasopharynx to 50 percent lung through this particle size range, and we just don't know what it is, so that the target tissue could vary from the nasopharynx and nasal carcinomas to the lung, depending upon what the real-life aerosol is that humans are exposed to.

So I would make a plea for good characterization data to develop information on potential exposure environments to which man or animals may be exposed and good deposition retention data to assist in determining dose to critical tissue. Both are key elements in estimating risk to human health. In adddition, it is important to determine the relative potency of hydrocarbons that are associated with diesel particles and to determine the elution rate of hydrocarbons from the particles and thus be able to estimate dose to critical tissues. Only by having all of these elements can one really conduct a defensible human health risk assesment.

DR. FLAMM: The gentleman from API has indicated--did you still want to comment or has that been taken care of by Dr. Jones' remarks?

DR. HOLDSWORTH: I guess I made the comment a little early, but I would like to respond and kind of second the gentleman from Chrysler's plea here that we get to the real world again. Apparently, my comments weren't heard.

With no disrespect to Dr. Cortesi, he seemed to think that we could extrapolate from one type of particle from another, and that's exactly what I said we can't do.

From a scientific point of view, yes, soot contains polynuclear aromatics as do all of these combustion products, but we don't know the nature of each one of them.

I think maybe we ought to take a look. It is no surprise to me, or I don't think anyone else, that all these things contain polynuclear aromatics, some of which are potentially mutagenic, potentially carcinogenic, et cetera, and they are extracted and they can be demonstrated to show activity.

But, I don't think that really reflects the relevant real-word situation. I think we do need to think about deposition particle size and all that, but I feel it can be handled from an inhalation standpoint even more practically than that. I think we can generate diesel emission in some sort of an inhalation setup and look at the results. It may take a lot of dollars, and a couple of years to study, but I think it can be done. I think the technology is there.

DR. FLAMM: Dr. Campbell?

DR. CAMPBELL: I guess it is time to say a little something about the inhalation toxicity business in Cincinnati, again; the gentleman missed yesterday's presentation.

We are, of course, conducting inhalation studies in Cincinnati, and the message seems to be getting across quite clearly that this near real-world type of study situation would benefit a great deal from this kind of dosimetry determination that has been discussed both yesterday and today.

We are going to be making an attempt to define or somehow get at what kind of organic materials are on our particulate material. We do have some histological evidence of deposition, albeit quite nonquantitative, and we have histologic evidence that this particulate material does indeed deposit in the deep lung, and is taken up by macrophages.

Macrophages are laden with particles that stay around for a long, long time, even after the termination of exposure, and they are found in draining lymph nodes.

Of course, we don't know, although we see particulate material there, how much has been eluted or remains included on the particle material. We don't know where it is eluted, or how much is getting to what target cells there are.

So, we do need some information of this sort, and I would welcome any suggestions as to how we might go about this with very limited personnel resources.

The methodology for tracking an index, if you will, perhaps a fluorescence index of some kind might be in order but I don't know, and I would defer to the chemists who might indicate to us a good method that we could incorporate to get some useful dosimetry information of this kind.

DR. ZEIGER: Zeiger. Could I ask you, are you looking at ingestion of these particles in chamber?

DR. CAMPBELL: No.

DR. ZEIGER: Because you've got a chamber filled with grooming animals, and there might be quite a considerable load taken up on that matter into the stomach.

DR. CAMPBELL: There is another aspect of the dosimetry that I knew would come up. I think it came up yesterday as well, or has come up in groups, anyway. No, we do not.

We realize this and, of course, it would be nice to be able to do head-only exposures if we only wanted to look at the inhalation exposure route. We can't do that in a long-term situation, of course.

DR. ZEIGER: I think the point I am driving at is that as long as you've got these animals in the chamber, you might as well look at as many endpoints and at as many parameters as possible, and at as many tissues as possible, and not just the lungs and the draining lymph nodes.

DR. CAMPBELL: Well, we are looking at the pathology of other tissues, but we are, of course, concentrating on the primary target, or which we feel would be the primary target.

We are looking for evidence of damage, or toxicity, in other organs or systems, as well, but we have to concentrate on the lung. The matter of dosimetry and ingestion of material, be it from the ciliary elevator clearance mechanism, or from grooming, we should look at because this, again, raises the question of what kind of methodology we can use so we can get useful information, even if it is only an index of some kind of the important materials that we are talking about.

Since specific chemical identification is questionable, and we don't even know which ones we should be looking for precisely, we are going to have to relate to indices of some kind, and we will welcome suggestions as to how we might do that.

DR. FLAMM: Yes, please?

A VOICE: Richard Schreck, GM Research. I have a question for the Cincinnati people, and that is: Is there food present in the chambers during inhalation? And if so, what is the rate of turnover of that food?

DR. CAMPBELL: Yes, it is, and we recognize that the palatability of the food could be a problem inasmuch as it might take up diesel exhaust just as well as animals can by absorption, or the particles might by adsorption.

We try to change the food on a daily basis, particularly in the exposure test chambers to get around this problem. They have feed and water available at all times.

We are only exposing 8 hours a day, of course, and they are serviced at the times when the test exhaust is not piped to the chambers, but the food is there and we're attempting to get around it by changing it frequently.

We have not noticed any effect of that kind as yet. We have noticed a depression in food consumption in a group of behavioral animals. These are rats. But we don't feel that this is because of a palatability problem.

DR. FLAMM: I suppose the foods are pellets rather than powder?

DR. CAMPBELL: That's right.

DR. FLAMM: Would the representative from GM care to say anything at all about their current or planned efforts?

DR. PEPELKO: I just want to make a short comment on this. I am Pepelko from Cincinnati.

I would just like to comment on the feeding of the animals: We feed them between 3:00 and 5:00 each day, and since most of these animals are nocturnal-type animals, we assume that they do most of their eating at night.

The engines are operated during the daytime, so our guess is that they probably don't ingest too much contaminated food.

DR. FLAMM: Yes, sir.

A VOICE: Jaroslav Vostal, GM Research, Detroit, Michigan.

I would like to respond to the invitation by the chairman that anyone who has been involved in studies on the potential health effects of the diesel particulates should come and tell the audience what has been done.

It is difficult for me to do it in a brief period of time, limited by the discussion period here. We are being supported by a corporation which is planning to put hundreds of thousands of diesel engines on the market in the year to come, and even more in the future years.

Obviously, it would not be responsible for the corporation to make this type of decision unless they have assured themselves that they are not preparing any excessive risk for the general population of the United States.

We have been, therefore, deeply involved in studies on potential health effects on the exposure to the diesel about the same period of time as the EPA group has been.

We have had the benefit to communicate, although it was not too frequently, with the groups at EPA, and we have already been able to express some of our concerns to them in our previous meetings.

We have been very pleased, and we would like to congratulate EPA on how much they have improved the general program on the health effects since the first time that we have met. We have been very pleased that some of the points that we have raised early in our discussions have been accepted and been implemented in a very perfect way, and probably better than even we could do it.

The general concern about wider use of the diesel engine started about 3 years ago and, obviously, this was a time when we didn't have any other choice than to go into literature, and try to prepare a literature survey on how we stood with the knowledge on the health effects of diesel particulates at that time.

We have reviewed all the available literature, and we have been able to identify not only enough data on the characterization of the diesel particulates, but also some previous studies on the biological activity of the particulates. What was even more important, we have been able to discover that there have already been some studies done in the world, e.g., epidemiological studies, and that the most important one is the study of the London Group of the Medical Research Council which has been satisfactorily discussed here.

When we analyzed the data, we found that we can concur completely with the comments by Dr. Seltser: In spite of some reservations which any epidemiologist would have about these studies, it is a positive feeling to see that a group of experts in epidemiology in the City of London, where the epidemiology has had such a long tradition, prepared a study showing that in spite of excessive exposures, going up to the levels of 1,500 micrograms or 3,000 micrograms per cubic meter, in spite of the exposures which can be counted in the thousands of the manyears, in spite of the stable population working there for 25 years, there has not been any possibility so far to determine any excess in the frequency of the lung cancer among people exposed to diesel emissions for many years.

Obviously, this was a very encouraging message but we have, in spite of it, also developed a very extensive experimental program. We have initiated studies on the mutagenesis of diesel particulates about the same time as Dr. Bradow has got his data from the Southwest Research Institute, and from the Stanford Research Institute.

We have found complete agreement with him as to the findings of similar effects. The point is only how to interpret this type of the data, and this is the point where we started to diverge and to differ in opinions with EPA.

We think that the studies which have been done by EPA so far and, unfortunately, which have been temporarily stopped, are very important. As we have seen, they have done excellent studies on the characterization of the components of diesel particulates responsible for the mutagenic effects.

Again, we think these are very important studies and should be continued. The question is only how relevant are the results of these studies for a public health officer who has to make a decision if, and under what conditions, the diesel should be widely used on the roads. Obviously, the first thing that the public health officer should ask himself would be how much of the material which is deposited on the particle will be released into the body. As I said, there have been a lot of discussions just recently pointing out the importance of this aspect.

We can hardly expect that we have in the body mechanisms comparable to such a powerful solvent like dichloromethane which would completely strip from the particle all hydrocarbons previously deposited on it.

Obviously, we have proposed, and that has been done by the group at EPA, as well as by our group, to do comparative studies using not only extraction by the powerful nonpolar solvents, but to go for solvents which might be more acceptable for the biological systems like dimethylsulfoxide, or to go for even more relevant extractions of diesel particulates, by the serum, by the synthetic biological fluids, e.g., saline, etc., and we have heard a lot of discussion about it.

It is a pity that we didn't have the possibility during the presentation yesterday to share with you some of our data indicating that if you express the mutagenicity of the dichloromethane extract as 100 percent, only if you switch to the dimethylsulfoxide you find less than 50 percent of that activity, and I am sure that Dr. Bradow can confirm that, as well. Moreover, when you use the serum, or simulated biological fluids, you hardly can detect any mutagenic activity at all.

Now, this is only one point. Obviously, there are literature references confirming what was mentioned just a while ago by Dr. Huisingh that there are concerns about the possibility of a long residence time of the particles in the respiratory system and indications that the particles could reach the lymphatic system or other sites of the body.

This is again another crucial point. However, considering the excessively high exposure conditions under which the data was obtained, I think that we are trying to play a role of the devil's advocate and to find whatever is possible to be wrong about the diesel particulates.

We are forgetting about the most important factor for the public officer in his risk assessment process: What is the level of the expected potential exposure of the general public?

I have been surprised that in spite of the fact that we have been talking about how meaningful are the data to be expected from the studies conducted by the EPA, that there has not been anything mentioned so far about what could be the concentrations of the particulates which he can expect at the roadside, in the street canyon, and you know, even in the urban or rural areas or somewhere else in the years to come.

It would be very difficult for me who has been working in the field of environmental and industrial toxicology for many years to make any judgment about the significance or meaningfulness of a biological experiment for the real world when I have three orders of magnitude difference in the exposures between the experimental conditions and the real world.

It is very difficult to say that we are trying to identify the potential health effects of the diesel particulates when we are exposing the animals to 6,000 micrograms per cubic meter, and all the atmospheric model studies conducted by EPA, as well as by our Environmental Science Department in Detroit, indicate that all we can expect will be somewhere of the order between 5 and 10 micrograms per cubic meter, expresssed as an annual average value.

It is also difficult to accept that there will be a long residence time of the 5 or 10 micrograms of the diesel particulates in the respiratory system, in view that we have a very powerful lung clearance mechanisms and they are working very well under low levels of exposure although they can be completely blocked by high exposures.

Even from the so far very limited experiments that we have been able to obtain in our studies, we can show you how different is the clinical picture and the lung histology in the animal which was exposed not to 5, not to 10, not to 20, but maybe as high as 10 times more, 200 micrograms per cubic meter, and you can hardly find any trace of the free particulates in the lung, even by intensive microscopic evaluation.

On the other hand, you expose a group of animals only for 2 weeks to concentrations as high as 6 milligrams per cubic meter, and you may see the situation which has been described by Dr. Campbell. You will see practically all macrophages laden with hundreds of particulates, and many of them dying directly inside of the alveoli. You can really even trace the particulates deposited in the lymphatic tissue.

But, is this really the situation we expect? We have been discussing at summer meetings in Houston, or later meetings with the EPA group from Cincinnati, the significance of their pilot experiments where they reported that only after 16 days of exposure to this high concentration, 8 hours per day, they have found a lung which was completely black, which was so stuffed with the particulates that you could hardly cut it with the knife.

We really don't know how to interpret this type of data, and to make them relevant to the concentrations which are expected at the roadside.

We feel, again, we would like to say that we have been tremendously impressed by the expansion and acceleration of all the projects proposed by EPA on this important issue of diesel particulates.

However, although this has been quite impressive to listen to all the project outlines yesterday, we feel that probably in our mind it is not enough. It would be very difficult to make any judgment on how significant the conclusions may be of the experimental research where we have heard that the most important approach to be used will be only the comparing of the relative potency of diesel particulates with coke oven emissions, cigarette smoke, and all the other carcinogens.

We have heard from Dr. Huisingh about their studies on the mutagenetic effects. Unfortunately, we didn't hear anything from Dr. Albert to tell us what he will do with the data when he finally gets it.

As you know, it is not very impressive to hear that even the chairman of the committee for the Cancer Assessment Group has to admit that all the conclusions that he is doing are probably having an accuracy range of about three or four orders of magnitude. It is difficult to understand that the Cancer Assessment Group accepts only one of the available models, that means linear extrapolation--one of the models, and we know how large were the discussions in the scientific world about the validity of these models.

In this respect, I would like to concur with many of the preceding speakers and to say that probably we should not be satisfied with the scope of the presented studies on the qualitative aspects of the potential biological effects of diesel particulates, only to add another important aspect of the quantitative evaluation of the potential toxicity of the diesel particulates.

The studies on the particle size distribution and characterization of the diesel particulates have been done in our research laboratories, and also by EPA. We have been able to show in our presentation in Houston, as we have heard also here today, that you cannot compare one diesel engine's particulates with particulates generated by another engine.

We have been highly critical about the original studies done by the EPA since they all have been based only on one single sample originally, which has been taken from the heavy-duty engine.

We know that there is a lot of discussion in the engineering world about the factor of how representative is the collection of the particulate samples on a filter, and how much the dilution factor can play a role. We would just like to add to the factors which were mentioned here by Dr. Bradow at least one other important parameter, i.e., temperature changes, and there may be many more that should be taken into consideration as well.

We have heard some concern about the artifacts occurring on the filters, and, you know, if we accept the existence of artifacts on the filters, where do we stand today and what is the validity of all data we have collected up to now?

All that has been done so far is to collect particulates on the filters. We have introduced another method of collection by electrostatic precipitation in trying to eliminate all these potential factors wherever it is possible and we strongly feel the necessity to do so also in the future.

We feel that one of the most important aspects which has not been considered enough so far is the retention of diesel particulates in the lungs, and the lung clearance of the diesel particulates. This aspect has not been even touched upon by the EPA program at all.

We completely agree with Dr. Jones, and many other previous speakers, that this is one of the crucial points. Maybe we will be surprised that there are no particles with a long residence time, there are no particles which will penetrate deeply into the tissues when the experimental exposures are kept as low as have been predicted for the year 1985.

As I mentioned before, we have been disappointed that we have not heard too much about the final process of the risk assessment process. We feel that Dr. Albert's presentation yesterday was very superficial, although this is probably the most important process in the entire evaluation of the potential health effects of diesel emissions. I feel strongly that the approach should be more relevant to the orders of magnitude of the expected concentrations in the field. All what I can say at this moment is that we would be glad to share with you the results of some of our few data. Obviously, we don't have such a large professional staff as EPA has, but we have accumulated data that are meaningful in the light of the expected exposures.

So, whoever is interested in what we have found, we are not hiding it, please come to Detroit. We would be very glad to share it with you. Thank you.

DR. FLAMM: Thank you very much.

Dr. Cortesi indicated that he wanted to reply to some of your comments.

DR. CORTESI: I think there is a misconception here about what a Federal agency that is supposed to protect the public health has to do. I am rather surprised that at this late date we still hear fussing about going from high to low dose exposures, and how we do it in cancer assessment.

There is no question here. We are not trying to hide that that isn't a problem, or anything else. It may not be perfect science, but it certainly has been science that has been well tested in the courts and found not to be artibrary and capricious.

I think to come out and say that we can't do anything until we have found the absolute mechanism of cancer, how much elutes, and this and that, and nailed everything down, is rather childish, frankly.

It has been well tested in the courts and found not to be arbitrary and capricious, and I did not think Dr. Albert was being superficial.

He was honest in pointing out the problems that one has in predicting a risk, and I thought he made it clear that we were going to do comparative potency on the basis that he prescribed.

While that is not watertight science, it is simply good policy and not unscientific in the sense that it goes against the grain of what we would expect to be normal scientific policy. Thank you.

DR. FLAMM: Did you want to reply?

DR. VOSTAL: Yes, but Dr. Bradow wanted to comment on the issue. Did you?

DR. BRADOW: Yes. In Dr. Vostal's comments, several fairly--really very interesting points were brought up, and many of these we have some degree of agreement with.

With regard to the nature of the experiment that, I think, has been presented that I have heard here in the last 2 days, it appears that the health effects research program is primarily aimed at determination of the relative potency of materials associated with diesel exhausts.

The matter of exposure assessment which Dr. Vostal has brought up is an extremely important issue and, in fact, there are issues associated with exposure above and beyond isolated exposures to diesel particles alone which, I think, are very, very important also.

Let me point out that we have had experience in a variety of mobile-source problems in the past several years where exposure assessments to areas, particular exposures from transportation-related pollutants have been worked previously, for example, sulfuric acid issues from passenger cars, and we have a substantial amount of modeling information contributed very largely by Dr. Chock of General Motors Research, and others on the General Motors staff, which has been very helpful in EPA to allow us to calculate these particular isolated exposures.

One characteristic of mobile-source pollutants generally is that their incremental concentrations tend to be high in relatively isolated situations. For example, the roadway itself. Generally speaking, the roadway itself is the largest exposure.

The question of time of exposure on that roadway is a very important issue, I think. The matter of exposure to transportation-related pollutants above and beyond the areawide exposures to, say, particle emissions from stationary sources is also a very important issue.

We are not looking at the exposure of individuals to diesel particles in the void of other exposures, and probably, in my view, of other exposures to similar chemicals, similarly carbon-bound from combustion sources other than automobiles. When stationary sources of various types burn the same fuel, chemicals are probably evolved which are very similar to those which are found in diesel particles.

Your point is well taken, Dr. Vostal.

In the matter of attempting to establish what sort of regulatory policy is necessary for the Environmental Protection Agency, we, I think, in research and development are very concerned and hope to be able to produce suitable exposure assessments for use by the regulatory arm of the agency for weighing this risk against other risks, possibly of greater magnitude.

DR. FLAMM: Thank you. Yes?

DR. VOSTAL: I would like to respond very briefly to what Dr. Cortesi said.

I certainly have not had any legal education, so I hardly can say what is arbitrary and capricious. I would like to leave it for the appropriate court actions to do it.

On the other hand, I feel that the meeting which we attend here is a meeting of scientists--of the concerned scientists who would like to find out what the best method is to evaluate a potential risk coming from the introduction and the wider use of the diesel engines on our roads.

Obviously, the most crucial point of this concern is what are we looking for or what are our objectives. If I am allowed to comment only about one fraction of the very extensive EPA program presented to us during the last 2 days, I would like to talk about the skin painting.

You all know that there have been voices in the past which question the relevancy of skin painting for lung cancer, or the validity of the application of nitrosourea into the trachea as an experimental model for lung cancer.

Is the model really appropriate, or is it a model which doesn't give us anything more than the fact that we have at least some tool in our hands to produce changes in the tracheal mucosa?

These are very crucial questions which have to be raised in connection with the planned research if we want to make a sound and meaningful decision. It is difficult to accept that all we will be doing is a study on the relative potency, comparing the coke oven emissions with the diesel extracts, with the cigarette tar and some other carcinogens.

We will try to evaluate it by stripping off all material that is deposited on the particles, and somebody was even talking about analyzing the entire particle, and to test it on the skin, although we know that the possibility that this will happen to the same extent in the biological system is very limited. Therefore, what I would propose is that even in spite of the fact that Dr. Keeve mentioned to me that the only part of the program concerning the skin painting is funded at the level of $470,000, that maybe what we need is another $470,000 for studies where skin painting should be done with the whole particulates left in close contact with the skin, or should be done with the extract of the particulates obtained with more biologically relevant material than dichloromethane.

Obviously, there are many other aspects of it which can make the studies more relevant to the relative biological potency of the diesel particulates and which need to be discussed. It makes no sense to spend time now to discuss only if we should expose 25 cats, or 50 of them, to discuss relatively minor technical details, and at the same time to ignore the basic principle, how will we interpret the data accumulated within 6 months, or 2 years from now?

DR. FLAMM: I think there is a need to have an understanding of the relationship that may exist between apparent exposure, human exposure, and what the true dose is, and considerations of what elutes, and in what timeframe, and what not, is extemely important to that.

Whether skin painting studies can help in that regard, it is hard to say, but I think with the help of Dr. Jones and others on the panel, we are really going to need to address that question which I, personally, think is a very important one.

It is true that those who have faith in toxicology, and toxicologists, and faith in animal models, and so forth, are or do on occasion out of necessity operate in the face of those uncertainties and ambiguities; and one of the reasons, as I understand it, that the EPA has proposed these multiple comparisons is to provide for some indication of validity in what it is doing.

If it had really wanted to just accept the data at face value, this entire thing could have been streamlined down to issues relating only to the diesel fuel emission, and I think the general overall philosophy--I don't know exactly how it is going to be applied--does provide for some indication as to the validity of the data, and Dr. Albert was in essence saying yesterday that he cannot detail the exact risk assessment approach until he sees the data and, at that time, he can begin to construct something that would be perhaps defensible.

DR. VOSTAL: Mr. Chairman, I apologize coming back to the microphone again, but I think there is at least one more comment which I would like to say.

The point that you have phrased just now is very important. We know that there is being circulated at EPA a draft of a document called "The EPA Policy on Airborne Carcinogens." It is really a pity that although I and many of my colleagues have been trying to get the possibility to see and read the document, there is no potential way you can get it unless you are a member of the select circle.

It is very difficult for us to say that we are discussing here as scientists such an important issue on the potential carcinogenicity of the diesel particulates, when all we can hear is that we don't have any policy really presented to us still, and the chairman of the Cancer Assessment Group says, "I would like to see first the data and then I will tell you what I will do with it."

I hope that you will agree with me that it is very difficult for me and all of us here to contribute in the discussion on the meaningfulness of the presented program if the responsible investigator admits, "I don't know what I will do."

This is one more thing which I would like to comment on and this is the reply which I got from Dr. Cortesi: He said that I called the treatment of the risk assessment by Dr. Albert superficial but I probably will take the exception from his opinion and I will repeat, yes, it was superficial, unfortunately, although this in my mind was the most important issue of the day.

I don't know how we can apply an approach which is called relative potency when we do not know the mechanisms responsible for the effects. Now, can I offer to you some other potential model?

We know that the asbestos is a typical carcinogen and we have relatively good epidemiology data where we can compare these particulate matter with the diesel particulates.

If I ask Dr. Huisingh to compare both toxins in the mutagenicity testing, she will not find anything positive. Isn't it so?

DR. FLAMM: That is apples to oranges, in a sense.

DR. VOSTAL: Exactly. Now, what is really the similarity between coke oven emissions and between diesel particulates, and I would probably accept a little more similarity between the tar from the tobacco or other hydrocarbons, but I am still very uncertain even in this respect exactly what I wanted to find. Maybe we are comparing apples, oranges, and grapefruits, and I don't know how I will do it. Why not asbestos? Why not use vinyl chloride? Why not use anything else?

DR. FLAMM: I am sure the panel in its deliberations will consider the points that you have articulated.

DR. CORTESI: We may admit to comparing oranges and grapefruits, but certainly not oranges and apples. (Laughter.)

DR. FLAMM: I sense that we are all beginning to run out of gas.

Yes, sir?

A VOICE: Dr. Brooks from Lovelace Foundation in Albuquerque.

I was kind of hoping someone else would ask this question so I wouldn't have to. I would hate to have the panel go away from here thinking that a single point constitutes a dose response, and that the inhalation study as designed with a single point is adequate.

So, I want to make sure that these points are made. I'm sure that the people on the panel know this already. It seems a shame to me since the inhalation part of this whole exercise will probably be the most useful part in doing hazard evaluations, that it has been limited to a single exposure point.

If you were to take the same number of animals and expose them to two levels, wouldn't your information content be higher?

Or, if you were to take the same money and, instead of doing the multiplicity of tests proposed, concentrate on the inhalation exposure, your hazard evalution be better.

DR. FLAMM: Yes, go ahead.

DR. CORTESI: I think it might be helpful if I reiterated a part of what Roy Albert said yesterday about the way that the Cancer Assessment Group extrapolates to low levels. As you heard him say, it is a linear no-threshold extrapolation which only requires one point to get at those responses, and we are not claiming that we are doing that buttressed by science.

We are doing that as a matter of agency regulatory policy, and it is a policy decision to do that and not a scientific decision, and the minute science tells us a better way to do it, we will adopt it with an alacrity that will leave you breathless.

DR. BROOKS: Two data points would be a lot better. Even with linear extrapolation, I think it is necessary to know more than a single data point. I think if it is a policy decision it is a lousy one. (Laughter.)

DR. CORTESI: You'd better propose a better one.

DR. BROOKS: Two data points is a better one; okay?

DR. CORTESI: Not necessarily.

DR. BROOKS: Very much so. If, for example, you are exposing animals at a very high level where you have a lot of interaction, a lot of cell inflammation, your dose response may come up very sharp, go out, and you get one data point and run it back, and you are way, way off.

On the other hand, it may go out very, very flat, go right straight up. If you have two data points you at least know whether it is going to go down, or go across.

You would double--you would more than double your information content with the same expense.

DR. FLAMM: Would you identify yourself, please?

DR. BENJAMIN: Fred Benjamin, National Highway Traffic Safety Administration.

I think it is a good thing if you compare various carcinogenic substances like cigarette smoke and diesel exhaust. However, the basis for comparison has to be a logical one. In this case, I think the basis should be human exposure, and not something arbitrary like the maximum possible dosage. If the evaluation is based on time and concentration of human exposure, I think you might obtain more meaningful results.

DR. FLAMM: Yes, Dr. Church?

DR. CHURCH: Charles H. Church, U.S. Army.

I want to put this whole thing in perspective. You people forget you are really playing with dynamite. The rule that Dr. Cortesi has to deal with, from what I can gather, is so strong that if you can find evidence of cancer caused by diesels, EPA is required by law to prohibit the introduction or at least--

VOICES: No, no.

DR. CHURCH: Excuse me, to prohibit any waivers upon the NO_x rule; isn't that right?

DR. ZEIGER: No.

DR. CHURCH: Okay, let's say the rule is not strong and there is no such thing. (Laughter.)

If you put the rulemaking down at such a low level that essentially bans the diesel, then when you would go down to your local friendly automobile dealer and get out your little booklet with the EPA mileage, the cars that you find that you will be able to buy in 1985, unless there are massive breakthroughs, are going to be about the size of a Pinto or a Chevette. You say, "Well, I like those kinds of cars," but, from the Army's standpoint, it drives us into buying vehicles that are beyond the rulemaking (i.e., heavier than 8500 inertia weights).

You are really working into a very difficult area. I realize that EPA has got a very difficult problem, but we need to listen to the people from the industry, and we've got to have an element of reason.

We are concerned about the carcinogenicity in the human species as exposed to diesels, as they would be used on the highways and in the cities, and only that is the issue that is involved in the rulemaking; isn't that right, Roger?

DR. CORTESI: The zoo that occurs when we make rules is a different public meeting than this one, so I don't think I will discuss rulemaking at this one. Thank you. (Laughter.)

DR. HUISINGH: Joellen Huisingh at Research Triangle Park.

On the comparative studies, I think it should be remembered that it is proposed that these studies not only be conducted with organic extracts in the skin, and in those studies we do not presume that we are comparing the same biologically active material. We presume that we probably are not.

It is still reasonable to compare biological activity, at least of those organic--in those test systems. How you extrapolate that to whether those organics leave the particles is a different issue, but I would like to also recall to everyone's memory that the presentation on intratracheal instillation showed that whole particles will be introduced in those studies into the lungs, and there will be some comparisons with studies with the whole particle and not just with the organic extracts.

Possibly, it might be recommended, or I am presuming that a number of people in the audience are recommending, that we need to concentrate maybe a little more on the aspect which I think I raised, and a number of people from EPA did raise, on whether those biologically active materials in such particles as diesel particles leave the particle and get to the target site within the cell.

I think there might be more we can do within our program to address that combining in vitro assays, cytogenetic studies with the intratracheal instillation studies, and those animals will be exposed, and there will be urine available and other body tissues, blood, and presumably sperm, and I think that I certainly would appreciate some sense of recommendation from Dr. Vostal or the panel, if those parts of the program may need some expansion on to try to address this question.

The other way to address this question, I think, is to really move forward as fast as we can with Dr. Bradow's program to identify potential mutagens, because once we do that--or else characterize the fluorescent material adequately--we then can begin to measure exposures, even within humans, and we want very much to do that.

We can then look and see if those materials are bound to DNA, if they are--we can follow their release from the particles, and their distribution throughout the animals and use it as a monitoring tool in the human population.

This is as an important aspect of both current and future research. It is very hard for us, I think, to predict right now with the demands on the program how soon we could make progress in this area, and what impact it could have on decisions this fall, but it is certainly something we would hate to see dropped from the program.

DR. FLAMM: Thank you very much, Dr. Huisingh. Dr. Goldman has a comment.

DR. GOLDMAN: Marvin Goldman, University of California.

At the risk of perhaps being a devil's advocate, I am concerned about this--speaking of risk--a risk assessment that is being asked to be produced in something of the order of a 1-year term.

This, of course, is going to hinge on what we are all hearing known as quality of the data base. The data base that now exists with regard to the potential health effects of diesel fuel has not been clearly elucidated.

I, as a panelist, am uncertain as to the extent to which the world's literature has really been evaluated to determine its deficiencies before embarking upon these tests, and I will play it a bit further: Assuming that there are going to be extracts that are going to be proven to be carcinogenic and mutagenic to some reasonable extent, and they may be comparable to other compounds that have been tested, we are dealing with an organic problem, and I have heard only that we are concentrating on the particulates.

It may well be that in comparing it with coke oven work data that we have overlooked something that has to do with the escaping of vapors in the exhaust as well.

I have heard little about the possiblity of atmospheric chemical changes that will change the quality of the material from that which is now being tested, and also, as has been mentioned by several others, the lack of a metabolic model eliminates the possibility perhaps of finding that there are antagonistic rather than synergistic effects also operating the biologic system.

Some of these materials which in the pure state, and in a bacterial test system, may prove to be mutagenic, may in a more complex in vivo situation prove to be inactivated, altered, or metabolites of certain innocuous fractions may be, shall we say, heated up and become quite potent.

In this regard, perhaps, intraperitonal injections do provide an in vivo incubation, if you will, potential that may be quite useful.

I think though that one thing can be done in the coming year, and perhaps should be a parallel activity, has to do with an ongoing, shall we say, paper exercise on risk assessment which would say that perhaps the limited human epidemiology may be concentrating on the London Transport Study which might represent an upper bound.

In risk assessment, I happen to agree with Dr. Cortesi that it is important to know what the quantities are, and what the slope is if you are talking about a linear model, and it is an academic exercise that is often frustratingly elusive as to what the mechanisms are that cause that to occur. To do a risk assessment though, it is not essential that you fully understand the complete molecular steps that go into the carcinogenic process, much as we would like to.

So, therefore, I would think that right now one could make an upper estimate of what that slope would be for humans, just taking the error term around the London Transport numbers as an upper bound to see what that slope is.

If this is inconsistent with extrapolation derived from a host of short-term bioassays, then the rather intriguing possibility of some rather significant metabolic alterations occurring must be understood.

I do not see how in the next 12 months we are going to be able to throw additional light on that, but with regard to risk assessment, the most important thing is to find out what the, shall we say, the exponent is in these numbers rather than what the coefficient is that precedes them.

If you can tell within an order of magnitude within a year what the problem is, I think we have come a long way. Then the regulatory process and the other policy decisions, which may not bear directly on the scientific interpretation, will know the ball park in which one is dealing, as to whether it is acceptable. If this is a one-in-a-million opportunity, or a one-in-a-hundred? At what point does something become less than innocuous and totally unacceptable? I do not know what the policy is.

One last point is that what is doable within a year might be an assessment of ambient air quality indices which are focused on organic separation technologies that are used for these diesel extracts. What is the extractable fraction in an urban canyon with whatever descriptors you wish?

I know there are studies already published on the fact that ambient urban air has proven to be mutagenic in some of these Ames Tests. These kind of comparators give you an indication of what background is, if you will, again with some of these other tests to be compared.

Unless that data base is available to you, we will be making estimates in a vacuum, and I think this can be quite hazardous.

DR. FLAMM: Thank you, Dr. Goldman.

DR. HUISINGH: Joellen Huisingh.

I didn't elaborate on the ambient studies since they are not primarily directed by OHEE. We are providing the bioassay support got the ambient exposure characterization studies. The currently planned studies will be conducted this spring in high and low diesel density areas. The ambient particles in the respirable size range will be fractionated, chemically characterized and bioassayed to determine if the presence of mutagenic activity or specific compounds is related to diesel emissions. Do you want to say more about that, Ron?

DR. BRADOW: Ron Bradow, EPA, North Carolina.

In addition to the studies that Joellen is alluding to which, in fact, are being carried out mainly by Bob Jungers in the EMSL Laboratory in Research Triangle Park, there is also a study which is already funded, and is planned now to occur during the month of March in the Environmental Sciences Research Laboratory.

There is a planned exposure in the Calspan smog chamber in Buffalo of diesel exhaust to a standard photochemical atmosphere. There is a standard photochemical experiment which is commonly run with propylene, a propylene experiment, which establishes the KE value for smog chamber.

Now, it turns out that with an FTP, a standard total emissions test with diesel passenger car, it is quite possible to get an appropriate level of hydrocarbon and NO in the smog chamber and, consequently, run a standard ozone radiation experiment which produces pretty much a full panoply, at least of the simpler gaseous molecules, in the presence of photochemical radiation.

So, this study is planned and, in fact, my group is providing to Joe Buffalini's group at Research Triangle Park the technical support to get the emissions experiment done, including the automobile, the test technicians, and the fuel. That particular study should enable us to find out something about the photochemical alterations.

Now, one of the big difficulties we have doing an ambient air experiment and, particularly, a standard photochemical experiment, is the mass of particle material present even in the extremely large chamber like the smog chamber at Calspan is very small, and it will require a number of experiments of that type even to supply sufficient material to run a very few Ames Tests, so in terms of expansion of the photochemicaly exposed material into the higher test, this may not be possible.

I think Joellen is planning for support to that experiment. Because of the very limited biological testing that is available, it is not a very substantial amount of work in the program.

A VOICE: Gerald Rausa, EPA, Office of Energy Minerals and Industry. I would like to respond to a number of comments or questions that have come up.

1. Dr. Flamm requested information pertaining to other possible activities that are going on that may bear on this subject. Essentially, in energy-related research, there are probably 1200 health-related projects going on at the present time throughout the country, and there is about $120 million being spent on the subject. So, to presume that Dr. Albert is going to use just this information on which to develop just the dose-response relationship is inappropriate.

2. It turns out there appears to be a lack of understanding of what the output of the health research activity is supposed to be, and it is my understanding that the output is an attempt to develop a data base to provide the dose-response relationship, not necessarily the hazard assessment itself, but rather risk. There is a difference between the two concepts.

The word "hazard" normally requires consideration of the source model, transport model, and the risk or dose response model. Essentially, this group concerns itself specifically with the development of the dose-response model. As a consequence, this group does need to interact with the other groups who are producing those other models.

3. With respect to the question of risk extrapolation, it appears that indeed one does have to know something about mechanisms in order to address the subject of extrapolation across species.

4. With respect to the linear nonthreshold dose relationship, the single-point extrapolation could very well be a lower bound from a very high-level exposure, and not an upper bound. That possiblity does exist, which essentially agrees with Dr. Brooks' contention.

I do want to emphasize the output of the risk group is not to produce a whole hazard assessment, but just to take the health data that is available, and not only that which is coming from this program but the totality, to try to develop the dose-response relationship.

DR. FLAMM: We are rapidly approaching adjournment. Before we adjourn, I want to call your attention to the fact that in addition to making comments from the floor, you can send comments to EPA, and that you will find the address for submitting those comments on a board immediately outside this room.

I would also like to thank this audience for patience, forebearance, and courtesy that it has consistently shown to the chair and the panel over the past 2 days, sometimes under rather difficult and strained conditions. It restores faith in human nature. So, we thank you very much for that patience and forebearance.

Let me just provide one more hopefully short opportunity for final comments. I believe that--yes? The gentleman from GM.

DR. VOSTAL: Since there has been something mentioned about what is the mission or objective of the health effects studies, and I really welcome the comment. I am sorry that it came practically at the end of our meeting. I think that a statement of the objectives should have really introduced the entire meeting.

If it is true that only the group at Cincinnati will provide a material for the dose-effect analysis, then I think that we should have some technical comments. As has been mentioned already here by our colleague from Lovelace, it is very difficult to consider one single exposure point as a dose-effect analysis study. I think that our colleagues from EPA will remember that at one of our earliest meetings we have proposed that our approach is to study several doses, and try really to extrapolate something from it.

If I am allowed to have a little positive criticism, I have to say that I was a little shocked yesterday since I have seen that there is a lack of communication between Roy Albert and the group in Cincinnati.

I was surprised when Roy Albert asked for the "maximum tolerable dose" as a model for the cancer, that the same project has been applied also to potential effects on the pulmonary function and many other things where it is not applicable at all. I feel that this could be improved, and the understanding between the group which will make the interpretation of the data, and the group which is collecting the data, if there could be a little more communication and understanding, I think that it will be beneficial for the entire program.

Just a last note: I really appreciated very much the comments by Dr. Huisingh when she asked for some positive input from the audience in proposing what should be done, and we would like to repeat again that this is what we have tried to do for a long time. Based on one of the early meetings between Mr. Costle and the representatives of the automotive industry, we have volunteered and we have been open for any type of dialogue where we could contribute whatever we could to improve the meaningfulness of any type of the studies, we are ready to do it. It was disappointing for us that it takes such a long time. We are certainly in favor of more frequent studies and more continuation of the dialogue. Thank you very much.

DR. FLAMM: Yes?

A VOICE: Stan Blacker, EPA.

I would like to be assured as a representative of the Program Office that the panel in its deliberations provide the Program Office with some critically important information as a result of this meeting.

In summarizing what has been presented yesterday, in my mind I have gone over what information might be available to the Program Office a year from now. They may only have relative potency information in the in vitro screening area and in the skin painting area. In the skin painting area, only papilloma data, and possibly only those skin painting experiments measuring tumor initiation. In the intratracheal instillation area, we might have information from the Schreiber method, possibly comparing other materials besides the diesel for relative potency. We might have results from the strain A mouse, from inhalation study and the intraperitoneal injection work with strain A mouse. My recollection that the Program Office may not have much else on which to base its decisions.

The thing that I am very much concerned about, and my office is concerned about, is the quality of the information that is going to be obtained, the overall approach, the quality of the models that are going to be used in taking this information, and the quality of the data and the ability to take that data and possibly extrapolate it to a human effect using the cancer assessment approach of relative potency.

I would like to have the panel, as a part its deliberations, to emphasize and reflect on these concerns. I am concerned by some of the discussions that have gone on that perhaps the panel may come up with a series of research recommendations for further work, as opposed to focusing in on one important component, as the Program Office sees it.

DR. FLAMM: I think this perhaps an appropriate time to indicate the panel's understanding of the charge before it, and that basically is what you articulated, Stan, that we are to look at the things that have been planned, and to try to the best of our ability to determine its relevance and impact on the question of risk to the public health.

In addition to that, it would seem to me that unless we find the program to be in every sense, in every way perfect, to propose such modifications, additions, or deletions, as are believed appropriate pursuant to the question, and in that way make some contribution to the decisionmaking that the regulatory program is going to have to cope with, unless there are additional burning questions, comments, and I see a couple of hands that went up--would you identify yourself, please?

A VOICE: My name is Holmquist of the American Mining Congress.

I was interested in the observation that this panel plans to interact in its deliberations not only with other organizational components of HEW, but with other governmental departments, which I assume includes the Departments of Defense, Transportation, Interior, Labor, and so on, including the research work they have been doing in this same area.

I would be interested in some expansion on this point--just how this will work and what other departments you will be working with.

DR. FLAMM: Well, that I think is somewhat the mechanism by which the other Federal agencies interact, and is really beyond the purvue of this particular panel.

Someone from EPA would have to address procedures and mechanisms they plan to use or provide for that interaction. I don't question for a moment the importance of the questions you are asking, but simply its appropriateness to this meeting, and I would think that given the fact that that is going to be a long reply, and I am not sure that there is anyone here who is empowered to be definitive at this time regarding that mechanism, I beg your forgiveness and I will pass on to Dr. Campbell who indicated he wanted to make a comment.

DR. CAMPBELL: I will be very brief. I don't think we in Cincinnati will argue with anyone else on the value of multiple-dose studies, and of low ambient realistic level studies.

We landed on our single level by consultation with CAG under the emphasis that was given to this area of study effects interest. We could possibly have built in other levels, but to do so would have reduced our chamber capability for studying some other endpoints other than oncogenicity-related. That remains the case.

It is not impossible to shut down and reengineer the system to do this sort of thing, nor would it be impossible to attempt to do a very, very low level study on that sort of thing.

Of course, everybody, I think, in the room recognizes the reason why we are at elevated levels. We won't go into that. But, in order to study the multiple levels, we would have to shut down, there would be a delay, and we would not get back again with data of any kind for the fall of 1979 deadline, and this is the situation we are in right now. It can be done but we won't have any data, and to do so on multiple levels for oncogenic-related endpoints we would, indeed, eliminate space that we could use for some of the other supplemental information.

DR. FLAMM: It is quite apparent that in these instances, and in many instances where public policy decisions have to be made, that the scientists, and the investigators, and the planners are going to have to make a host of compromises to bring desires within the realm of reality which involves a host of constraints.

The thing that obviously we have to be careful of is that in making the compromises we don't get the wrong answers and wind up doing more harm than good. A responsibility of this panel, it seems to me, is to look exactly at that issue, and to make recommendations in accordance with that.

Again, I would like to thank the audience for its cooperation, and also for its contributions to these proceedings, and I, personally, look forward to seeing you again sometime. So, with that I would like to adjourn the meeting. (Applause.)

(At approximately 11:30 a.m., the above proceedings were concluded.)

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Appendix A
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Appendix B
ADDITIONAL COMMENTS
SUBMITTED AFTER THE MEETING

Your file Votre référence

Our file Notre référence
Pr. No. 4.3.6.1.02.01

December 19th, 1978.

Mr. Brent Norton
GEOMET, Incorporated
Gaithersburg, Md. 20760
U.S.A.

Dear Mr. Norton:

Thank you for the informative package on "Diesel Emission Health Effects Research". The [illegible]osed EPA work will inevitably have considerable impact on the underground use of diesels, on which the mining industry in both of our countries is heavily dependent. Therefore, I have prepared the enclosed submission as a "Written Comment" to ensure consideration of factors of significance to the mining industry.

Sincerely,

Paul Mogan

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and Energy Technology,
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"Written Comment" - Diesel Emission Health Effects Research

J.P. Mogan and E.D. Dainty

Projected EPA diesel research will inevitably have considerable impact on the underground mining industry because of its current heavy reliance on diesels as a power source. It would, therefore, be informative if EPA's sub-acute animal exposure studies bracketted current underground levels. Gaseous pollutant concentrations at 13:1 dilution ("Toxicological Assessment of Diesel Emissions" S.D. Lee et al - 71st Annual Meeting APCA, Houston, June, 1978) are about double those encountered underground (NIOSH Workshop on "The Use of Diesel Equipment In Underground Coal Mining" - Morgantown, September 1977; "The Development and Application of Advanced Mine Air Monitoring Techniques to Mines Using Diesel Powered Equipment", Johnson J. et al, Michigan Technological University, Feb. 1976). The 30:1 dilution ratio proposed for the "Chronic Diesel Studies" (pages 57 and 71 - Diesel Exhaust Emission Research Program) does therefore bracket current underground exposures.

It is also noteworthy that the 13:1 dilution ratio yields a particulate level of four times rather than twice the average underground concentration, due to the impact of engine derating for underground use. Since "underground rated" engines provide a versatile and efficient power package with only a slight weight per horsepower penalty, it is hoped that this parameter will be one of those investigated "to reduce or eliminate positive responses to the Ames system". (EPA's Program to Assess The Public Health Significance of Diesel Emissions", page 9) and will also be taken into account in the design of the sub-acute animal exposure studies.

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John L. Swift, Vice President
GEOMET, Incorporated
15 Firstfield Road
Gaithersburg, Maryland 20760

Dear Mr. Swift:

I have received word that your corporation is responsible for the proceedings of the Scientific Review meeting on Diesel Emission Health Effects Research, EPA, December 12-13, 1978, Arlington, Virginia.

The enclosed memo to Dr. Roger Cortesi is self-explanatory. I would be pleased if this is inserted into the proceedings.

Thanks very much.

Sincerely,

Robert B. Reger, Chief,
Epidemiological Investigations Branch

Enclosure

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

TO : Dr. Roger Cortesi
Chairman, Mobile Source Resources Committee

DATE: December 15, 1978

THRU: Director, DRDS
NIOSH, Morgantown JM

FROM : Chief, Epidemiological Investigations Branch
DRDS, NIOSH, Morgantown

SUBJECT: Comments relating to Scientific Review Meeting on Diesel Emission Health Effects Research, EPA, December 12-13, 1978, Arlington, VA

Although I attended the Health Effects Research meeting in Arlington, I was unfortunately unable to be present the morning of December 13 to provide comments for the record. It was stated at the outset of the meeting that the EPA would allow written comments to be inserted into the record. This is the purpose of this memorandum.

The overall charge at the meeting related to research dealing with health effects associated with light duty diesel engines. Further, the inferences to be drawn from the research will undoubtedly eventually relate to the human species. Lastly, the major organ system of the body considered paramount in this research involves the respiratory tract; and the disease entities of most concern are cancer of the respiratory tract as well as chronic non-carcinogenic lung disease such as emphysema, bronchitis, and obstructive airways disease.

I feel that the overall objectives of the research are sound. However, it is clear that the vast majority of the research which will be done by (and for) the EPA would be in vitro and in vivo studies, which in the main involve animal experimentation. The "bottom line" relating to EPA standards for health effects associated with light duty diesel engines must include significant input from epidemiological studies whereby the effects in the human species can be documented. Without question, there are significant shortcomings in the research approach presented in this area. At the present time, to construct an epidemiological study which is community based with an aim towards separating effects from diesel emissions from all other confounding variables is at best extremely difficult, and of course, at worst, impossible. This is especially true as it relates to light duty diesel health effects. Epidemiological studies of this nature are less complicated when they involve exposed occupational groups. Accordingly, it is my feeling that the epidemiological input on this subject will come from occupational as opposed to community based settings. In that most occupational groups who have been exposed to diesel emissions have had this exposure from heavy duty engines, inferences must be in an indirect fashion as they relate to exposures from light duty vehicles.

As opposed to some panel members it is not my feeling that on-going epidemiological research in this area is irrelevant to the charge of the EPA.

Before I give comments regarding our own (in-house) research in this area, I must comment on the weight the EPA (and a couple of panel members) seem to be giving to the London Garage Workers Study. Most epidemiological studies have some limitation associated with them. In my opinion, the London Garage Workers Study is fraught with such limitations that it makes the data totally unusable. It is not necessarily the case that just because we know the limitations involved that we can take account of them and temper our inferences accordingly. Dr. Miday listed no less than five serious limitations to this mortality study but by far the two most serious relate to no information on smoking habits and the fact that the cohort consisted of those within an extremely narrow age range. The fact that exposure data is meager is of concern and this is a problem with most studies of this nature. However, one should at least have an indirect exposure index and the London study does not.

Presently, at the Appalachian Laboratories in Morgantown, West Virginia, we are involved in several studies which are at least partially concerned with the effects of diesel emissions on the health of specific occupational groups:

(1) A study of metal/non-metal miners in the United States has been conducted. Over the years, heavy duty diesel engines have been used in the workplace where the workers were employed. In addition to medical examinations of the workers, MSHA performed detailed industrial hygiene studies at the same operations to characterize the atmosphere. On the basis of their data, company records, and occupational histories from the miners, exposure indices (limited in nature) were developed. These indices were related to health parameters; viz. pulmonary function, symptomatology, and radiographic appearances of chest diseases, at the time of examination. No consistent relationships were found between the health characteristics of the miners and their estimated exposure to diesel contamination in the workplace. A serious limitation in this study involves the fact that out-migration was not considered (drop outs), and results from the group studied may involve a survivor effect.

(2) A mortality study involving a cohort of over 13,000 metal miners examined in the late 1950's and early 1960's has been conducted. The cohort was followed through 1976 for mortality experience. There were no apparent differences in overall death rates or cause specific death rates for those who worked in diesel as opposed to non-diesel mines and occupations. This declaration, however, suffers from the limitation that the total diesel exposure of the men in the cohort was not known and could not be totally determined from their occupational history. It relies heavily upon their occupation and the mine in which they worked at the time they were examined in the prior morbidity study.

(3) As a subset of study #1 mentioned above, detailed examinations were conducted on salt miners exposed to varying concentrations of diesel contamination. This work force was essentially non-mobile. In addition to ascertaining chronic health effects amongst this group, before and after shift pulmonary function studies were also performed while personal environmental sampling was performed during the work shift. Changes in pulmonary function were associated with dose of NO_2. It cannot be concluded, however, that this dose response relationship is totally the result of diesel usage, as blasting (also producing NO_2) occurred in the workplace.

(4) A morbidity study relating to coal miners exposed to diesel emissions has been conducted. The study involves three separate components and as might be expected, has been designed specifically to answer questions relating to the health effects of coal miners exposed to diesel emissions. The three subparts are as follows: (a) chronic health effects; (b) acute health effects; (c) a special study of both 'a' and 'b' as it relates to women working underground in diesel exposed areas. Preliminary results are available for subparts 'a' and 'b'. In the main, the results show no consistent effects amongst miners exposed to diesel emissions; i.e., when compared to controls in the same industry who were not exposed to diesel emissions.

At the present time we cannot establish from the gravimetric sampling, the difference between diesel soot and ordinary coal mine particulate. However, in a qualitative sense, both are similar in character and both have the property to allow for adsorption of toxic gases which can be carried to the gas exchanging portions of the lung. Hence, whether or not a potentiating effect exists between the toxic component of diesel emissions and carbonaceous particulate can be investigated. This seems highly relevant to the charge given the EPA.

(5) Without going into specifics, I should state that both morbidity and mortality studies are either on-going or soon will be in relation to workers in railroad running repair shops as well as metropolitan bus garages. Moreover, similar work is being conducted (with our input and technical assitance) by the San Juan (P.R.) Health Department.

I mention all of these studies because I feel they are relevant to the overall picture of health effects due to diesel emissions. I dispute the implication at the meeting that current and proposed epidemiological work regarding diesel emissions in the mining industry and other industrial settings is generally irrelevant to the EPA's charge. At this time, any results mentioned in the above studies are to be considered preliminary. Publication will be in the near future.

Current epidemiological input to this important subject must not be dismissed, and the results from such studies should be the "bottom line" on standard setting in this area.

I would be pleased if this is inserted in the record of the December 12-13 EPA meeting which was held in Arlington, Virginia.

Robert B. Reger

December 27, 1978

Mr. Brent Norton
GEOMET, Inc.
15 Firstfield Road
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Re: AMC Diesel Committee comments to the December 1978 draft entitled "Diesel Exhaust Emissions Health Effects Program Protocols", the Mobil Sources Research Plan, and the health literature and evaluation document entitled "Health Effects Associated with Diesel Exhaust Emissions".

Dear Mr. Norton:

By notice in the Federal Register of December 5, 1978, (43 F.R. 56934), the Environmental Protection Agency (EPA) invited interested persons to submit to you comments regarding the EPA diesel emissions health effects research plan.

The Diesel Committee which submits these comments is a standing committee of the American Mining Congress (AMC), a national trade association whose members are the producers of most of the nation's metals, coal, and industrial and agricultural minerals, and manufacturers of mining equipment and machinery.

The Diesel Committee of the American Mining Congress has concerned itself with this subject for more than two years and welcomes the opportunity to comment on the EPA plan for diesel emission health research as reviewed at the December 12-13, 1978, meeting held in Arlington, Virginia. Our specific comments are attached hereto and made part hereof.

In submitting these comments, we respectfully protest the short period of time allowed for comments and the lack of adequate notice provided for the December 12 and 13 meetings. The notice of meeting appeared in the Federal Register on December 5, 1978. Interested parties, particularly on the west coast, did not receive the Federal Register until the date of the meeting. It is most regrettable that a topic as timely and important as the health effects of diesel exhaust emission was planned with such a limited lead time that optimum public response and participation was prevented.

Sincerely,

Richard C. Holmquist
Director
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American Mining Congress Diesel Committee Comments on the Environmental Protection Agency Diesel Emission Health Effects Research Program

While considerable thought appears to have been given to this research program, it also appears that adequate deliberation has not been taken with regard to the interpretation of the data that will be generated from this research if an objective decision regarding the health risks associated with diesel emissions is to be made. It is unclear just how the data from this research program will be used in making final decisions regarding the use of light-duty diesel engines and their subsequent health effects.

A central theme of the EPA documents describing the research program, which was reflected during the discussion in Arlington, Virginia, on December 12 and 13, 1978, was the desirability of determining the relative carcinogenicity of extracts from diesel exhaust particulates versus other pollutants such as cigarette smoke and coke oven emissions. Many uncertainties are recognized, which are admittedly vast. A question is thus raised of the desirability of the Environmental Protection Agency's attempting to make such crucial decisions on the basis of preliminary data. As was stated in the EPA document entitled "Health Effects Associated with Diesel Exhaust Emissions" the assessment of public health risks from diesel exhaust exposures cannot be done on the basis of animal data alone. Yet, contrary to this logic, the EPA is planning to render a decision based primarily on animal data. One of the major weaknesses which we feel is inherent to this EPA program is the lack of emphasis on human population studies. Another point that needs emphasis is the measurement and quantification of ambient levels of diesel exhaust and an accurate comparison of these levels to animal studies. Since the uncertainties in the present data plus the lack of time, resources and money to eliminate these uncertainties will not change dramatically before the need for an early decision by the EPA regarding the diesel conversion program, it is apparent that the decision cannot be one that is based on hard scientific evidence. Accordingly, the American Mining Congress is extremely concerned that the EPA will make this decision on the sole basis of the "linear, no threshold" risk assessment methodology. It would be most tragic to eliminate an option for energy conservation such as is represented by diesel fuel unless there is a clearly demonstrated public health reason for doing so. Such decisions, which can never be fully objective, should at least be made in terms of criteria that are commonly defined.

The following discussion will outline various points on the specific references mentioned at the beginning of the letter. With respect to the paper by Barth and Blacker entitled, "EPA Program to Assess the Public Health Significance of Diesel Emissions," our comments are as follows:

1. The statement that diesels emit more than 30 to 50 times the particulate load in comparison to catalyst-controlled gasoline engines is misleading. While the American Mining Congress does not refute this statement, we would like to point out that comparing a controlled gasoline engine with an uncontrolled diesel engine is obviously unfair and unscientific, particularly when a very large number of vehicles on the road are not catalytically-controlled and emit quantities of particulates equal to those of diesels. Control technology, when applied to diesel engines, offers a great deal of promise. Should one want to objectively compare the relative merits of diesel versus gasoline engines, it would be better to compare a diesel engine to the non-catalytic gasoline engine already in trucks and a large proportion of the passenger car fleet;

2. It was mentioned that EPA deemed it necessary to issue a news release on diesels solely based on the Ames test results. We find this bewildering in light of the fact that Amest test data has found urban air and emissions from gasoline powered vehicles to be mutagenic. No mention of these facts was included in this EPA press release. In our opinion the press release failed to give the reading public a balanced and responsible view of the subject it addressed;

3. It is our contention that it is not stressed strongly enough that a meaningful assessment of diesel exhaust exposures can only be made in conjunction with data on background exposures.

With respect to the Mobile Sources Research Plan dated October 11, 1978, our comments are as follows:

1. We would like to congratulate EPA on their constructive plans as iterated (starting line 152) to identify control technologies to help eliminate the harmful components of diesel exhaust;

2. From the discussion of studies (beginning line 301) to to evaluate teratogenicity, it would be helpful to do comparative testing of related pollutants as well. As has been identified many times, numerous substances are teratogenic, and to access these hazards in diesel exhaust exposures necessitates comparisons with similar exposure factors which currently are tolerated in the general environment. In the reproductive studies it would be helpful to be aware of and define the concentrations of diesel exhaust being used. Wherever possible a range of concentration would be more ideal;

3. In the behavioral studies, as with other studies, we would recommend that a range of concentration be given to assure an accurate assessment;

4. We would like to point out that NIOSH presently is doing several epidemiological studies, two of which were reported recently concerning exposures of diesel exhaust among miners. Within the next two years NIOSH will publish results of four mortality/morbidity studies concerning diesel exhaust exposures;

5. From the section on control technology techniques, no mention was made of the progress underway to design filters for mine applications of diesel units which are having some success. Such studies are currently underway under support from the Bureau of Mines.

The next series of comments concern the Diesel Exhaust Emissions Research Program draft dated December 1978. These comments are as follows:

1. More consideration should be given to the exhaust control metholology development currently underway and the anticipated development of particulate control devices which would greatly reduce the potential health effects related to diesel exhaust exposures;

2. We applaud the suggestion on page 7, where it is stated that a scientific review workshop would be held to evaluate this program after a six month milestone.

The American Mining Congress also recommends that such a workshop include participants from the various other government agencies and departments that are involved in this issue;

3. The approach that toxicity of diesel exhaust should be considered in relation to other pollutants is realistic and commendable;

4. With respect to development and application of microbial mutagenicity bioassays as a bioanalytical tool, we would like to recommend that such techniques, once they are developed and demonstrated to be reliable, be used to evaluate control techniques if and when pollutants of major health concerns are identified. In this manner, the effectivensss of pollution control devices could be demonstrated.

5. On page 49, where the test of carcinogenicity using strain A mice is discussed, there is no attempt to define the exposures to the test groups. It is critical to document and quantify diesel exhaust exposures in terms of critical species such as particulates, oxides of nitrogen, and so on. Unfortunately, no mention of doing this was made. Additionally, it would be helpful to have a graduated series of exposure concentrations to evaluate the possibility of a dose response curve;

6. On page 60, regarding the study on pulmonary function and pathology in cats, no mention of quantifying diesel dose exposures is made. Again, it is hoped that a range of doses could be used. The quantification of diesel exhaust exposures should also be undertaken;

7. In the discussion of the experiments on both pages 63 and 66, no mention is made of the concentration of diesel exposures. It would be helpful not only to know these exposures, but it would also be beneficial to compare these exposures to similar concentrations of gasoline exhaust;

8. In terms of behavioral tests on the rat, it is unclear how this data can be used in terms of judging the health significance. How does EPA propose to evaluate this in terms of other exposures? Additionally, on page 71, it would again be helpful to quantify the dose parameters as discussed previously and add another set of controls for gasoline exposures, as it would be most helpful to have gasoline exposures for comparison;

9. On page 74, where it is mentioned that gasoline extracts and skin painting studies have caused tumor formations, it is unclear how this information will be compared and utilized with reference to the skin painting tests with regard to the relative potency of diesel exhaust components;

10. On page 75, where the Battelle studies are discussed, the American Mining Congress would like to point to a major study weakness being the phenomenon of recovery periods similar to work or environmental exposures was not accounted for in that study protocol. We recommend that consideration of such research be undertaken. Secondly, the particulate (soot) levels used in the Battelle studies are perhaps excessive compared to those experienced in either mining or ambient environments. With respect to the infectivity model, it is puzzling that a gasoline control group is not included. Again, it is difficult to see just how this data can be interpreted in a meaningful way with respect to the infectivity model and the relative public health impact of diesel exhaust compared to gasoline exposures;

11. The time constraints involved for the recommendation by EPA on light-duty diesel vehicles will not allow for adequate data accumulation sufficient to allow solid scientific decisions. As such, if a decision must be made by mid-1979, this decision should not be final in lieu of more reliable data that will be available in the future. An alternate and more logical approach would be to delay a decision of this magnitude until sufficient information exists from which to draw reliable conclusions.

The remaining comments, which concern the EPA document entitled, "Health Effects Associated with Diesel Exhaust Emissions," are as follows:

1. It should be pointed out that diesel exhaust, which contains presumptive carcinogens as defined by the Ames test, tested similarly to the exhaust of gasoline powered engines and the ambient environment in major U.S. metropolitan areas;

2. More emphasis should be placed on the use and development of control devices which have been identified to reduce the emphysematous lesions in dog lungs and the instance of bilateral renal sclerosis in rats as a result of gasoline engine exposures;

3. Assessment of the public health risk due to diesel exhaust exposures cannot be done on the basis of animal exposures and therefore a greater emphasis on epidemiology must be stressed. While the document clearly points this out, a lack of general emphasis on this point seems to be inherent throughout the EPA program. Certainly greater efforts can be undertaken to evaluate occupational groups such as cab drivers, truckers, engine repair men, underground engine repair personnel, and miners exposed to diesel exhaust exposures;

4. This document very persuasively points out that there is no way currently to put the hazard of diesel exhaust in a proper perspective to enable an accurate assessment of the public health risks involved. Accordingly, it is most unfortunate that the EPA deems it necessary to make a solid committment to such a decision by mid-1979.